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BIOCHEMICAL NITROGEN FIXATION STUDIES

I. EVIDENCE FOR LIMITED OXYGEN SUPPLY WITHIN THE NODULE

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(WITH SIX FIGURES)

Introduction

During the last four years various types of studies dealing with the metabolic changes occurring in legume nodules have been carried on at this laboratory. Some of the more interesting phases of the work already completed will now be reported in a short series of articles, of which this is the first. The primary purpose of all the work was to obtain fundamental information on the biochemical processes taking place in the nodule as a basis for a better understanding of the nitrogen fixing process occurring there. The information at present in the literature dealing with nodule metabolism is extremely meager.

The present paper presents evidence concerning the oxygen supply within the nodules. The general experimental methods used are also described briefly. Special techniques employed in certain phases of the work will be described in connection with the particular results concerned.

[1] The writers are indebted to ELLEN K. RIST of this laboratory for the nitrogen analyses and to E. C. BUTTERFIELD of the Bureau of Plant Industry for supplying much of the plant material.

Methods

The general method used in most experiments was to culture detached nodules or roots for a few hours in a culture solution in a given atmosphere, and record the data desired. Usually the nodules were maintained in Warburg respiration vessels that were constantly shaken in a water bath at 28° C., and oxygen consumption or both oxygen consumption and carbon dioxide production measured as desired. In some cases, to be discussed in later papers, these data were supplemented by total gas analyses and special chemical determinations. Total nitrogen was determined by the usual Kjeldahl method.

The legume species used to the greatest extent in these investigations included: cowpea (*Vigna sinensis* (L.) Endl.), crown vetch (*Coronilla varia* L.), Korean lespedeza (*Lespedeza stipulacea* Maxim.), hairy vetch (*Vicia villosa* Roth), common vetch (*Vicia sativa* L.), soybean (*Soja max* (L.) Piper), crotalaria (*Crotalaria spectabilis* Roth), and sweet clover (*Melilotus alba* Desr.). Other plant species used in some of the investigations will be given in later papers.

Except for crotalaria and crown vetch, the plants just listed are common legumes, and it is therefore unnecessary to describe their nodules. The crotalaria nodules are of the vetch type and usually become branched after the first few days of growth. Crown vetch is not a true vetch although the leaves resemble hairy vetch to a marked extent. Its nodules resemble those of vetch except that they are usually less branched.

The legumes, with some exceptions, were grown in the field at the Experimental Farm at Arlington, Virginia. In most cases plantings were made at intervals in order that nodules of the desired type would be available when needed. Ordinarily only young active nodules from plants between the ages of two weeks and prior to flowering were used.

In many phases of these studies it would have been advantageous in successive experiments to use nodules from the same plant species that were of approximately the same age and in as nearly the same physiological condition as possible. This is obviously impossible when the nodules are grown under ordinary field conditions and experiments are conducted over a considerable period of time, since,

as our experience has repeatedly shown, the condition of nodules on a given plant may change markedly within a period of less than a week.

In the absence of standard or uniform nodular material, we chose to have growing at all times several species of legumes from which we could on any day select the nodules which seemed best suited to the experiment planned. One of the important results of the use of this procedure has been to show in a general way that, in so far as our observations extend, nodules of all legume species behave very much alike. For instance, if a given energy source is used by one type of nodule, as evidenced by an increase in the rate of respiration or an effect on respiratory quotient, nodules from other legume species will almost certainly also use the same substance. Nodules from different species may show quantitative variations but qualitatively they nearly always respond similarly. In fact, variations between different batches of nodules of the same species are ordinarily of the same order as those between different species, assuming that in both cases only nodules comparable in size and condition are used. Verification of these statements will be found in the tables included in this paper. It is desirable to emphasize these points in the beginning since frequently successive experiments have been made with different species of nodules.

PREPARATION OF NODULES.—The plants were dug carefully in the morning between the hours of 7:30 and 9:00, quickly transported to the laboratory, and washed. The nodules were picked off, again washed, sorted from defective ones (including all that floated in the wash water), dried between filter papers, weighed, and placed in the culture vessels. During most of the 1–4 hours that usually elapsed between the time of arrival of the plants at the laboratory and the beginning of the experiment, the nodules were kept in ice water or in the refrigerator at about 5° C.

The surfaces of the nodules were not sterilized before they were introduced into the respiration vessels, since in preliminary studies the few methods of sterilization tried reduced the rate of respiration appreciably.

When crushed nodules were used the method was to weigh out the usual quantity of nodules into the respiration vessels and crush

them with the blunt end of a glass rod. The nodules were not thoroughly ground up, therefore, but only broken and most of the contents squeezed out. The outer portions of the nodules, when of a tough structure, remained more or less intact as empty shells.

MANOMETRIC PROCEDURES.—When oxygen uptake and carbon dioxide production were measured, the usual procedure was to place 50–250 mg. (usually 150 mg.) of the fresh nodules in each Warburg vessel. Two cc. of basal medium containing the source of energy (if any), and usually without a nitrogen source, was then pipetted into the vessel. The basal medium described in detail elsewhere (1) was the same as used for the culture of rhizobia, and had the following composition: K_2HPO_4, 0.8 gm.; KH_2PO_4, 0.2 gm.; NaCl, 0.2 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; $CaSO_4 \cdot 2H_2O$, 0.1 gm.; $Fe_2(SO_4)_3 \cdot 9H_2O$, 0.01 gm.; and H_2O (distilled) 1000 cc. The two phosphate salts were dissolved first and a solution of the other salts added. The medium was allowed to stand for several hours until the precipitate had settled out; then the clear solution was siphoned off as needed. Nitrogenous salts were not added except in a few cases mentioned. Glucose was supplied in about half the experiments, as shown in the tables. If oxygen consumption only was to be observed, 0.3 cc. of 2N KOH was placed in the center well of the vessel. In the few cases where samples of tissue greater than 200 mg. were used, half of the alkali was placed in the center well and the remainder in one side arm in order to increase the surface and thus aid rapid CO_2 absorption. When, in addition to oxygen consumption, the carbon dioxide produced was to be recorded, the latter was absorbed in 0.4 cc. of saturated $Ba(OH)_2$ solution placed in one of the side arms. In the other side arm 0.7 cc. of 2.5 N HCl was used. At the close of the experiment the hydrochloric acid was dumped first into the barium solution, decomposing the barium carbonate, and then into the nodules, stopping metabolic processes. During early work only 0.3 cc. of 2.5 N HCl was used, but it was found that with this amount of acid, evolution of carbon dioxide after acidification continued at a decreasing rate for considerable time. This difficulty in obtaining a sharp end point was overcome with only occasional exceptions by the use of the larger quantity of acid. Since the standard details of these

manometric procedures are given by DIXON (2), as well as by numerous other investigators, they need not be repeated here.

Repeated tests showed that experiments could ordinarily be carried out under non-sterile conditions for periods of 5–6 hours without bacterial action becoming significant. The respiration data reported in this and subsequent papers of the series are based upon periods of 2–6 hours, usually 3–5 hours. Oxygen consumption was recorded at intervals of 0.5–1 hour during the experiment, whereas carbon dioxide values were necessarily for the entire period.

ENERGY SOURCES.—During the early stages of these studies some eighteen energy sources, including mostly sugars, organic acids, and alcohols, were tested in a preliminary way for utilization by nodules. The ability of the plant tissues and bacteria to use these was judged by the effect on rate of oxygen consumption and respiratory quotient, but in many cases the results were neither clearly positive nor negative. Of the sugars used, glucose, as might be expected, seemed to give the best results and was used for practically all the work where a readily available source of energy was desired. A concentration of 1 or 2 per cent was usually employed.

It was found that nodules usually show about 80–95 per cent as high a rate of oxygen uptake in the absence of added glucose or other energy sources as in their presence. Hence "endogenous" respiration tends to mask the effect of added substrate and makes it difficult to determine manometrically whether it is utilized.

SIZE OF NODULES.—No very exact expression of nodule size is used in presenting the data. Nodules are designated merely as very small, small, medium, large, and very large. In using these terms cowpea nodules were considered as an approximate standard. If the diameters are expressed in millimeters the terms have the following approximate values: very small, 0.5–1.5; small, 1–2.5; medium, 1–4; large, 4–6; and very large, above 6. When small or medium sized nodules were used, the large ones were discarded but not the very small ones. This accounts for the wider spread in size in these two grades. Since some nodules, such as those of hairy vetch, are irregular in shape and branched, it is difficult to classify them; such large branched forms were discarded. The smaller young forms are

classified according to their approximate average diameters. The small nodules of the branched type often included some pieces of the larger ones.

The symbols used throughout this series of papers are defined as follows:

Q_{O_2} = ml. O_2 consumed per mg. dry weight of tissue per hour
$\dot{Q}_{O_2}$ = ml. O_2 consumed per mg. moist weight of tissue per hour
$Q_{O_2}(N)$ = ml. O_2 consumed per mg. tissue nitrogen per hour

$$\text{R.Q.} = \frac{\text{ml. } CO_2 \text{ produced}}{\text{ml. } O_2 \text{ consumed}}$$

Experimental results

During the course of these investigations about 700 Q_{O_2} and 350 R.Q. determinations have been made to date, as well as a considerable but smaller number of other types of analyses. It is obviously impossible to report all these experiments in detail, hence the usual procedure will be to group together the data bearing on any point, no matter from what experiments secured. In many cases an experiment planned primarily for one given purpose may furnish excellent data on other points. This is one of the advantages of the standardized Warburg respiration technique. Ordinarily averages of two or more determinations, rather than individual values, will be given.

The rates of respiration of nodules are reported on the bases of moist weight, dry weight, and nitrogen content, except when the necessary data for calculation of some of these values were not obtained. In the discussion, reference will ordinarily be made to the Q_{O_2} values. The $Q_{O_2}(N)$ values are of secondary interest in connection with the present study but are included for the sake of completeness. Special reference is made to these values in the second paper of this series.

Rates of respiration and respiratory quotients of whole nodules maintained in various partial pressures of oxygen in nitrogen

The data given in table 1 are taken from numerous experiments run at various times during the 4-year experimental period. A

TABLE 1

RATES OF RESPIRATION AND RESPIRATORY QUOTIENTS OF DETACHED NODULES FROM SEVERAL SPECIES OF LEGUMES MAINTAINED IN VARIOUS PARTIAL PRESSURES OF OXYGEN

Experiment no.	Plant species	Size of nodules	Glucose (%)	O_2 in N_2 (%)	$\acute{Q}_{O_2}$	Q_{O_2}	$Q_{O_2}(N)$	R.Q.
11	Hairy vetch	Medium	0	21	0.563	5.63	.....	1.01
11	Hairy vetch	Medium	1	21	0.625	6.25	.....	1.00
51	Hairy vetch	Small	0	100	0.915	7.29	88.4	1.08
85	Hairy vetch	Very small	1	7.4	0.286	2.31	25.8	.....
85	Hairy vetch	Very small	1	21	0.526	4.24	47.4	.....
85	Hairy vetch	Very small	1	50	0.897	7.23	80.8	.....
85	Hairy vetch	Very small	1	100	1.108	8.93	99.8	.....
86	Hairy vetch	Small	0	100	1.220	8.16	108.5	1.11
86	Hairy vetch	Small	1	100	1.260	8.43	112.0	1.24
87	Hairy vetch	Small	0	21	0.570	3.83	57.7	1.15
87	Hairy vetch	Small	1	21	0.580	3.85	58.0	1.44
13	Crown vetch	Medium	0	21	0.281	.....	.....	0.97
13	Crown vetch	Medium	1	21	0.342	.....	.....	1.03
15	Crown vetch	Medium	0	21	0.357	2.03	33.1	1.23
15	Crown vetch	Medium	2	21	0.407	2.31	37.7	1.30
16	Crown vetch	Medium	0	21	0.380	2.41	35.0	1.15
16	Crown vetch	Medium	2	21	0.384	2.44	35.4	1.24
16	Crown vetch	Medium	0	100	0.797	5.06	73.5	1.08
17	Crown vetch	Medium	0	21	0.424	2.54	38.3	1.05
17	Crown vetch	Medium	2	21	0.447	2.68	38.9	1.09
18	Crown vetch	Medium	2	21	0.382	2.71	34.8	1.08
19	Crown vetch	Large	2	21	0.248	1.57	20.0	1.26
19	Crown vetch	Small	2	21	0.404	2.54	32.5	1.13
23	Crown vetch	Medium	2	21	0.304	1.91	24.4	1.17
23	Crown vetch	Medium	2	100	0.407	2.56	32.8	0.89
83	Crown vetch	Small	1	100	1.170	7.13	82.2	.....
84	Crown vetch	Small	1	100	1.210	8.29	100.9	.....
27	Common vetch	Small	0	21	0.347	2.80	38.5	1.00
27	Common vetch	Small	0	80	0.783	6.32	86.9	0.97
27	Common vetch	Small	2	7.4	0.210	1.69	23.3	1.30
27	Common vetch	Small	2	21	0.428	3.45	47.5	0.98
27	Common vetch	Small	2	50	0.711	5.73	79.0	1.01
27	Common vetch	Small	2	80	0.943	7.61	104.7	1.03
27	Common vetch	Small	2	100	0.970	7.82	107.7	1.02
65	Crotalaria	Small	0	100	0.749	5.74	72.0	.....
65	Crotalaria	Small	1	100	1.020	7.82	96.8	.....
66	Crotalaria	Small	0	21	0.353	2.30	29.2	.....
66	Crotalaria	Small	0	50	0.602	3.91	49.8	.....
66	Crotalaria	Small	0	100	0.813	5.28	67.2	.....
67	Crotalaria	Small	1	21	0.406	2.93	33.8	.....
67	Crotalaria	Small	1	50	0.711	5.13	59.3	.....
67	Crotalaria	Small	1	100	0.944	6.82	78.7	.....
70	Crotalaria	Small	1	100	0.971	7.25	86.6	.....
78	Crotalaria	Very small	0	100	0.873	6.19	69.7	0.95
78	Crotalaria	Very small	1	100	1.121	7.95	89.6	1.04
36	Sweet clover	Medium	0	100	0.652	3.83	85.7	1.03
36	Sweet clover	Medium	1	100	0.832	4.84	109.4	0.95
50	Sweet clover	Small	0	100	0.903	6.03	70.7	1.02
50	Sweet clover	Small	1	100	0.992	6.61	77.5	1.09
52	Sweet clover	Small	0	100	1.181	6.38	78.2	1.04

TABLE 1—*Continued*

EXPERIMENT NO.	PLANT SPECIES	SIZE OF NODULES	GLUCOSE (%)	O_2 IN N_2 (%)	$\bar{Q}_{O_2}$	Q_{O_2}	$Q_{O_2}(N)$	R.Q.
28	Lespedeza	Medium	0	21	0.281	1.69	22.8	1.17
28	Lespedeza	Medium	1	21	0.300	1.80	24.3	1.37
30	Lespedeza	Medium	0	21	0.269	1.58	19.8	1.11
30	Lespedeza	Medium	0	80	0.764	4.48	56.2	0.97
30	Lespedeza	Medium	0	100	0.855	5.02	62.8	0.94
30	Lespedeza	Medium	1	21	0.294	1.72	21.6	1.40
30	Lespedeza	Medium	1	80	0.875	5.13	64.3	1.03
30	Lespedeza	Medium	1	100	0.930	5.45	68.4	1.01
31	Lespedeza	Medium	0	21	0.257	1.50	19.1	1.26
31	Lespedeza	Medium	0	80	0.726	4.22	59.0	1.01
81	Lespedeza	Small	0	21	0.229	1.24	17.6	0.98
81	Lespedeza	Small	1	21	0.238	1.30	18.3	1.29
21	Soybean	Small	2	21	0.334	2.05	35.8	1.79
22	Soybean	Small	2	21	0.328	2.01	35.2	1.52
24	Soybean	Small	2	21	0.285	1.50	26.4	1.79
25	Soybean	Small	2	7.4	0.183	0.87	15.6	1.52
25	Soybean	Small	2	21	0.293	1.39	25.2	1.23
25	Soybean	Small	2	50	0.474	2.25	40.7	1.00
25	Soybean	Small	2	100	0.662	3.15	56.9	1.06
37	Soybean	Small	2	21	0.334	1.43	30.4	1.28
37	Soybean	Medium	2	21	0.301	1.28	27.3	.....
37	Soybean	Large	2	21	0.178	0.76	16.2	2.00
37	Soybean	Small	2	100	1.010	4.32	91.9	.....
37	Soybean	Medium	2	100	0.588	2.51	53.4	.....
37	Soybean	Large	2	100	0.288	1.23	26.2	1.16
38	Soybean	Small	2	100	0.967	4.19	88.0	1.03
39	Soybean	Large	2	100	0.501	1.98	45.1	.....
40	Soybean	Large	2	100	0.491	1.94	44.3	.....
41	Soybean	Large	2	21	0.153	0.62	13.4	.....
41	Soybean	Large	2	100	0.390	1.58	34.2	.....
42	Soybean	Large	2	21	0.248	1.01	22.7	.....
42	Soybean	Large	2	100	0.402	1.64	36.8	.....
1	Cowpea	Medium	0	50	0.394	.....	.....	.....
1	Cowpea	Medium	3	50	0.408	.....	.....	.....
6	Cowpea	Large	3	21	0.147	.....	.....	.....
49	Cowpea	Small	0	100	0.430	2.45	43.9	1.03
49	Cowpea	Small	1	100	0.488	3.78	49.8	1.04
55	Cowpea	Small	0	100	0.751	2.91	.....	1.03
55	Cowpea	Small	1	100	0.723	3.77	.....	1.00
56	Cowpea	Very small	0	100	0.600	3.24	.....	1.06
56	Cowpea	Very small	1	100	0.717	3.88	.....	1.11
57	Cowpea	Very small	0	100	0.703	3.74	.....	1.10
57	Cowpea	Very small	1	100	0.813	4.33	.....	1.06
68	Cowpea	Small	0	100	0.765	4.45	75.3	.....
68	Cowpea	Small	1	100	0.787	4.58	77.6	.....
71	Cowpea	Small	1	100	0.776	3.86	64.8	.....
75	Cowpea	Very small	0	100	1.050	5.80	100.2	1.06
75	Cowpea	Very small	1	100	1.153	6.37	110.0	1.10
79	Cowpea	Small	0	21	0.156	0.71	12.6	.....
79	Cowpea	Small	1	21	0.193	0.88	15.5	.....
80	Cowpea	Small	0	21	0.176	0.81	14.2	.....
80	Cowpea	Small	1	21	0.189	0.86	15.3	.....
82	Cowpea	Large	1	100	0.292	1.91	31.7	.....

typical set of time curves, drawn from data obtained in experiment no. 17 and showing the changes in rates of respiration during a 6-hour experimental period, is shown in figure 1. The effect of varying the partial pressure of oxygen upon the Q_{O_2} values of nodules is illustrated very strikingly in figure 2.

The rate curves in figure 1 illustrate some of the points already mentioned, particularly that glucose often has little effect upon the respiration of nodules. The curves also show that the rate of respira-

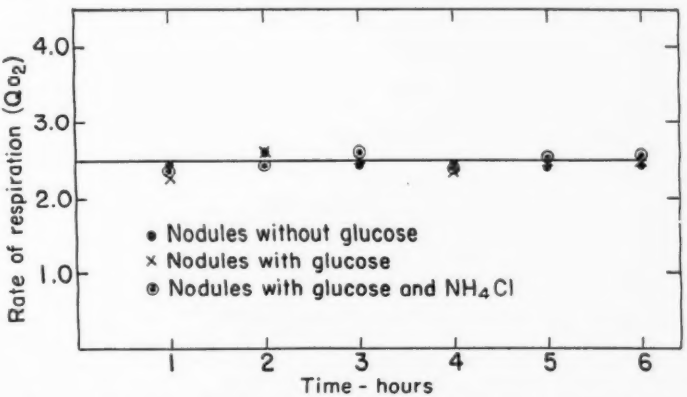

FIG. 1.—Rates of respiration of crown vetch nodules during 6-hour period maintained in various nutrient solutions in air.

tion of the nodules receiving ammonium chloride at a concentration of 100 ppm together with glucose was not significantly different from the rate with glucose alone. This observation was made in other experiments, hence combined nitrogen was omitted in all but a few of the respiration studies. It will be observed further that the respiration rate remains fairly constant over the 6-hour period of the experiment. If bacterial action had been an important factor, the respiration curve of course would have turned up sharply during the period of rapid bacterial growth. Occasionally the rate of respiration after a few hours will show a steady decrease instead of an increase. This decrease may sometimes be due to deficient energy supply in the nodules, but is more likely to occur when sugar is supplied, and when an increase in acidity occurs.

The Q_{O_2} values in air (table 1) varied from 0.62 to 6.25 for the

various plant species, with an average of about 2.2. The values in the presence of glucose, in the experiments where direct comparisons were made, were on the average about 12 per cent higher than in its absence. The young small nodules usually gave considerably higher

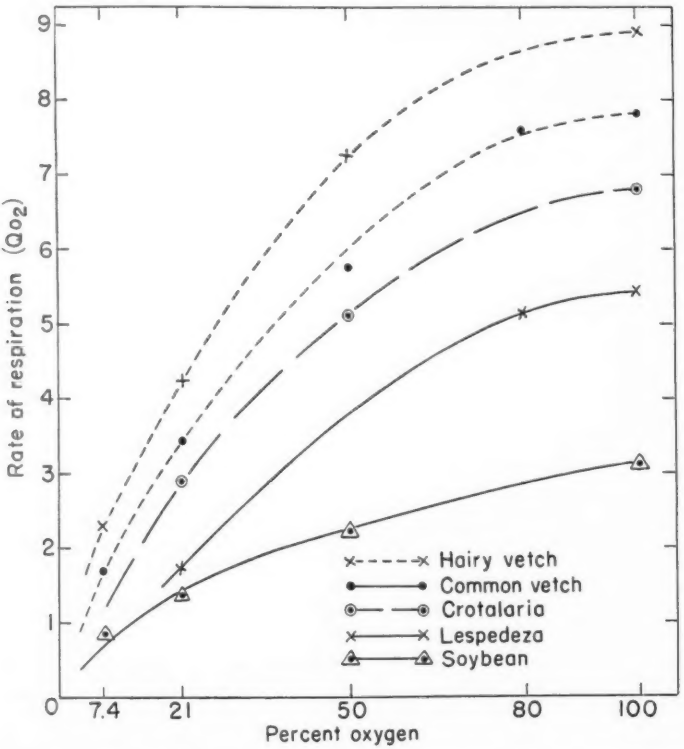

FIG. 2.—Rates of respiration of nodules from various species maintained in glucose medium in various partial pressures of oxygen.

values than the older and larger nodules, as would be expected. A relatively high or low value is usually not so much a characteristic of the plant species as of the size and condition of the nodule.

The Q_{O_2} values for nodules were markedly increased above the values in air by higher partial pressures of oxygen. An average of

the data from which figure 2 was drawn gives a Q_{O_2} of 2.75 in air and 6.43 in pure oxygen, an increase of 134 per cent even though only small or medium sized nodules were used. Likewise an average of all of the data in table 1, exclusive of the few experiments with large nodules, shows corresponding Q_{O_2} values for air and pure oxygen of 2.30 and 5.41, an increase of 135 per cent due to the additional oxygen. The addition of glucose to the nodules maintained in pure oxygen produced a mean increase in Q_{O_2} of 14 per cent over those given no carbohydrate.

The data of table 1 show that there is some tendency for the nodules of soybean, cowpea, and lespedeza to respire less rapidly than the nodules from the vetches, crotalaria, and sweet clover. This is probably due chiefly to the fact that the nodules from the first three species are usually less succulent and oxygen seems to penetrate less rapidly. If we compare the maximum, rather than the average, Q_{O_2} values observed for the nodules of the various plants as reported in table 1, the differences are somewhat less striking. The maximum Q_{O_2} values are for cowpea 6.4, lespedeza 5.5, and soybean 4.3; the values for the other plants are for hairy vetch 8.9, crown vetch 8.3, crotalaria 8.0, common vetch 7.8, and sweet clover 6.6. Apparently if sufficiently young succulent nodules are studied, the maximum Q_{O_2} values for all these species vary within a range of about twofold.

The respiratory quotients for nodules maintained in air and supplied with glucose show values ranging from approximately 1.0 to 2.0; in the absence of glucose the values were slightly lower (table 1). The theoretical R.Q. for the complete oxidation of glucose is of course 1.0. From these data, as well as from others not reported in table 1, it was noted that if the nodules were very small, young, and succulent, the respiratory quotients in the presence or absence of glucose were usually not far from the theoretical for glucose oxidation. When large nodules were used, however, the R.Q. values in air commonly reached a value of 1.7 or even higher. Such high values were most often obtained with cowpea and soybean nodules, owing in part to their larger size but also to the nature of the nodules. Apparently oxygen penetrates these nodules less rapidly than it does the vetch type of nodule.

The respiratory quotients, when much above 1.0 in air, usually show a marked decrease to near the theoretical value for glucose oxidation as the partial pressure of oxygen is increased. The R.Q. reaches a value of near 1.0 before the maximum Q_{O_2} is observed, indicating that even though the center of the nodule is not receiving the maximum amount of oxygen it is capable of using, the sugar is completely oxidized. In pure oxygen the R.Q. values, exclusive of those for the largest nodules, average about 1.03 and are not significantly different whether glucose was or was not added. In view of this and the fact that nodules are usually fairly well supplied with carbohydrates, it seems likely that glucose and probably sucrose, the more common sugars of most plant juices, are the chief energy sources used in normal nodule respiration. Of course other compounds often found in plant tissues have theoretical R.Q. values of near 1.0 and cannot be excluded as possible energy sources by the data presented here.

A number of factors influence the rate of oxygen consumption by plant and animal tissues. WARBURG (**2**, p. 50) early recognized the chief ones: (*a*) rate of respiration characteristic of the tissues being studied; (*b*) rate of diffusion of the oxygen through the tissues (which is a constant under a given oxygen pressure); (*c*) thickness of the portion of tissue used; and (*d*) concentration of oxygen immediately outside the tissue. It is obvious that in order to increase the oxygen supply inside a given nodule it is necessary to increase the partial pressure of O_2 surrounding it or to resort to slicing or crushing in order to provide mechanically for oxygen entrance. The use of smaller nodules, as already pointed out, also permits better aeration, but such smaller nodules naturally represent material biologically different from the larger ones.

DIXON (**2**, p. 50) points out that it has been determined in studies with liver maintained in air that the slices must be less than 0.2 mm. thick in order for the center to receive adequate oxygen; in pure O_2 the slices may be about 0.5 mm. in thickness. It is therefore not surprising that in table 1 most of the R.Q. values for nodules in air are above 1.0, even though few large ones were used. Even the smaller ones usually had a diameter of near 1.0 mm., and unless the diffusion constant of oxygen in nodular tissue is unusually high, or the

TABLE 2

RATES OF RESPIRATION OF CRUSHED NODULES FROM SEVERAL SPECIES OF LEGUMES MAINTAINED IN VARIOUS PARTIAL PRESSURES OF OXYGEN

Experiment no.	Plant species	Size of nodules	Treatment	Glucose (%)	Oxygen in N (%)	$\dot{Q}O_2$	Q_{O_2}	$Q_{O_2}(N)$
9.....	Hairy vetch	Medium	Whole	0	21	0.426	.....	.....
9.....	Hairy vetch	Medium	Whole	2	21	0.500	.....	.....
9.....	Hairy vetch	Medium	Crushed	0	21	0.525	.....	.....
9.....	Hairy vetch	Medium	Crushed	2	21	0.601	.....	.....
10.....	Hairy vetch	Medium	Whole	2	21	0.384	.....	.....
10.....	Hairy vetch	Medium	Crushed	2	21	0.501	.....	.....
85.....	Hairy vetch	Very small	Whole	1	7.4	0.286	2.31	25.8
85.....	Hairy vetch	Very small	Crushed	1	7.4	0.328	2.64	29.6
85.....	Hairy vetch	Very small	Whole	1	21	0.526	4.24	47.4
85.....	Hairy vetch	Very small	Crushed	1	21	0.384	3.10	34.6
85.....	Hairy vetch	Very small	Whole	1	50	0.897	7.23	80.8
85.....	Hairy vetch	Very small	Crushed	1	50	0.498	4.01	44.9
85.....	Hairy vetch	Very small	Whole	1	100	1.108	8.93	99.8
85.....	Hairy vetch	Very small	Crushed	1	100	0.472	3.80	42.5
83.....	Crown vetch	Small	Crushed	0	7.4	0.195	1.19	13.6
83.....	Crown vetch	Small	Crushed	1	7.4	0.222	1.35	15.6
83.....	Crown vetch	Small	Crushed	0	21	0.348	2.12	24.5
83.....	Crown vetch	Small	Crushed	1	21	0.338	2.06	23.8
83.....	Crown vetch	Small	Crushed	0	50	0.313	1.91	21.9
83.....	Crown vetch	Small	Crushed	1	50	0.483	2.94	33.8
83.....	Crown vetch	Small	Crushed	0	100	0.478	2.91	33.6
83.....	Crown vetch	Small	Crushed	1	100	0.522	3.18	36.6
83.....	Crown vetch	Small	Whole	1	100	1.170	7.13	82.2
84.....	Crown vetch	Small	Crushed	1	7.4	0.277	1.90	23.1
84.....	Crown vetch	Small	Crushed	1	21	0.360	2.47	30.0
84.....	Crown vetch	Small	Crushed	1	50	0.442	3.03	36.8
84.....	Crown vetch	Small	Crushed	1	100	0.484	3.31	40.3
84.....	Crown vetch	Small	Whole	1	100	1.210	8.29	100.9
65.....	Crotalaria	Small	Whole	0	100	0.749	5.74	72.0
65.....	Crotalaria	Small	Crushed	0	100	1.053	8.07	100.0
65.....	Crotalaria	Small	Whole	1	100	1.020	7.82	96.8
65.....	Crotalaria	Small	Crushed	1	100	1.140	8.74	108.2
70.....	Crotalaria	Small	Crushed	0	21	0.624	4.65	55.6
70.....	Crotalaria	Small	Crushed	1	21	0.733	5.47	65.4
70.....	Crotalaria	Small	Crushed	0	50	0.642	5.54	66.2
70.....	Crotalaria	Small	Crushed	1	50	0.815	6.08	72.7
70.....	Crotalaria	Small	Crushed	0	100	0.777	5.80	69.3
70.....	Crotalaria	Small	Crushed	1	100	0.921	6.87	82.1
70.....	Crotalaria	Small	Whole	1	100	0.971	7.25	86.6
9.....	Lespedeza	Medium	Whole	0	21	0.396	.....	.....
9.....	Lespedeza	Medium	Crushed	0	21	0.902	.....	.....
9.....	Lespedeza	Medium	Whole	2	21	0.391	.....	.....
9.....	Lespedeza	Medium	Crushed	2	21	0.924	.....	.....
1.....	Cowpea	Medium	Whole	3	50	0.408	.....	.....
1.....	Cowpea	Medium	Crushed	3	50	0.925	.....	.....
2.....	Cowpea	Medium	Whole	0	50	0.240	.....	.....
2.....	Cowpea	Medium	Crushed	0	50	0.524	.....	.....
2.....	Cowpea	Medium	Whole	1	50	0.224	.....	.....

TABLE 2—*Continued*

EXPERIMENT NO.	PLANT SPECIES	SIZE OF NODULES	TREATMENT	GLUCOSE (%)	OXYGEN IN N (%)	$\acute{Q}_{O_2}$	Q_{O_2}	$Q_{O_2}(N)$
2.....	Cowpea	Medium	Crushed	1	50	0.551	.....	.....
3.....	Cowpea	Medium	Crushed	1	50	0.760	.....	.....
58.....	Cowpea	Very small	Crushed	0	100	0.982	5.47	.....
58.....	Cowpea	Very small	Crushed	1	100	1.043	5.81	.....
71.....	Cowpea	Small	Crushed	1	21	0.733	3.64	61.1
71.....	Cowpea	Small	Crushed	1	50	0.964	4.80	80.3
71.....	Cowpea	Small	Crushed	1	100	1.116	5.55	93.2
71.....	Cowpea	Small	Whole	1	100	0.776	3.86	64.8
82.....	Cowpea	Small	Crushed	0	100	0.649	3.45	69.1
82.....	Cowpea	Small	Crushed	1	100	0.739	3.93	78.7
82.....	Cowpea	Large	Crushed	0	100	0.531	3.47	57.7
82.....	Cowpea	Large	Crushed	1	100	0.542	3.54	58.9
82.....	Cowpea	Large	Whole	1	100	0.292	1.91	31.7

Q_{O_2} values very much lower than for liver, the nodules must be somewhat deficient in oxygen in the center.

When pure oxygen is used and the nodules are very small (about 1 mm. in diameter or less) they are apparently rather well aerated, and nearly maximum Q_{O_2} values usually will be observed. The center of larger nodules maintained in oxygen will, however, undoubtedly be deficient in this element. These findings are in fairly close agreement with expectations from the observations with liver, considering that liver usually has a Q_{O_2} two or three times that of nodules supplied adequate oxygen. Since in some of these experiments a portion of the nodules used exceeded 3 mm. in diameter, it could not be expected that maximum Q_{O_2} values would be reached, even in 100 per cent oxygen.

RATES OF RESPIRATION OF CRUSHED NODULES MAINTAINED IN VARIOUS PARTIAL PRESSURES OF OXYGEN IN NITROGEN

It is obvious that crushing produced somewhat variable results upon the rate of respiration (table 2). In general the Q_{O_2} was increased by crushing if the nodules were maintained in air, owing of course to the better aeration of the cells. Even in 100 per cent oxygen, crushing commonly produced a favorable effect upon the rate of respiration, if the nodules were of medium or larger size or were

of the type that did not allow oxygen to penetrate readily. In the majority of cases, however, if small nodules were crushed and maintained in pure oxygen, a marked decrease in Q_{O_2} occurred. Figure 3

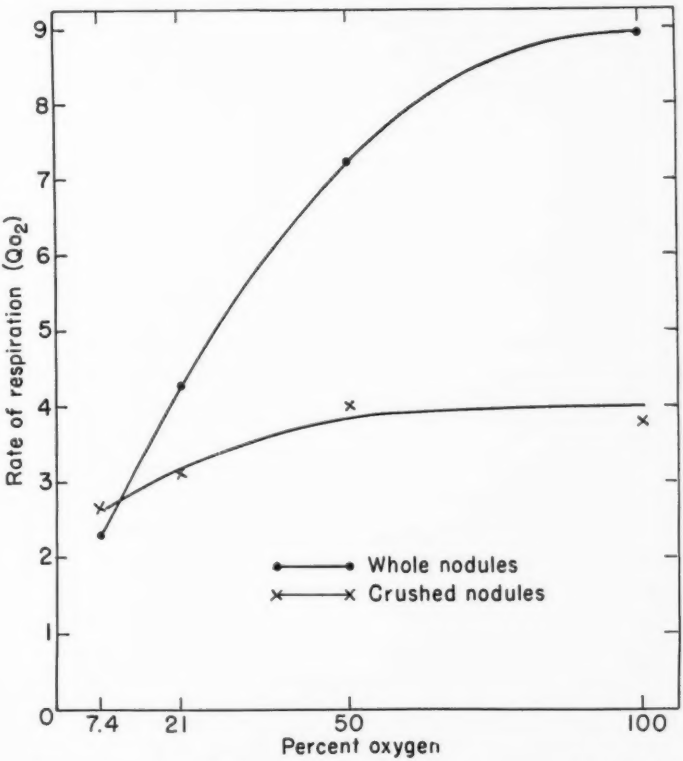

FIG. 3.—Rates of respiration of whole and crushed hairy vetch nodules maintained in various partial pressures of oxygen in nitrogen.

illustrates this point very well, although the harmful effect of crushing was unusually marked. A time curve showing the comparative Q_{O_2} values of crushed nodules in air and in pure oxygen, with and without added glucose, is given in figure 4. The decrease in Q_{O_2} during the first 3 hours was slightly more pronounced in pure oxygen. Evidently this method of increasing the aeration of the

inner cells of nodules is not very satisfactory if the observations continue for more than a few minutes.

The curves in figure 5 indicate that there is some stimulating effect of oxygen pressure, as such, on rate of respiration of crushed nodules. It would seem that such nodules would certainly be thoroughly aerated when constantly shaken in air. Nevertheless there was an increase of about 35 per cent in the Q_{O_2} values of such crushed

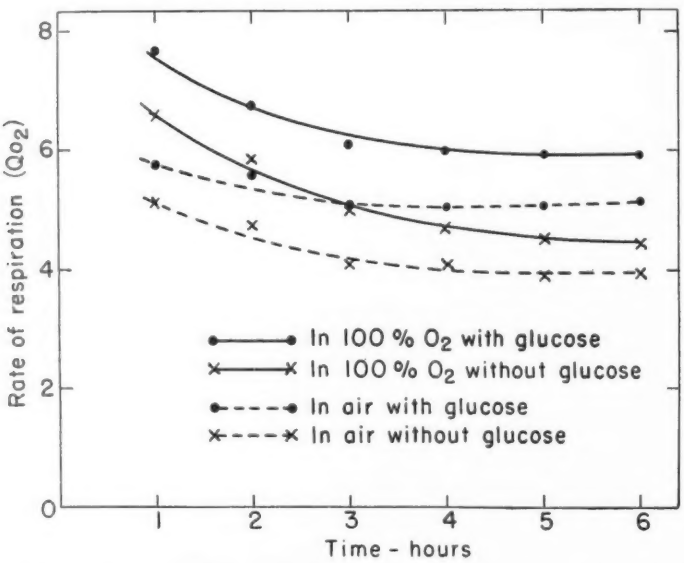

FIG. 4.—Rates of respiration of crushed crotalaria nodules in air and in 100 per cent oxygen in presence and absence of added glucose.

nodules when shaken in pure O_2 in comparison with the corresponding values for those kept in air. It should be remembered, however, that the nodules in these experiments were not completely macerated but merely broken and most of the contents squeezed out. Further experiments would be necessary to determine definitely whether the observed increase is a true oxygen concentration effect or merely an indication that even crushed nodules are still not adequately aerated for maximum oxygen consumption under the experimental conditions. The latter explanation seems somewhat more plausible.

Effect of glucose concentration on respiratory quotients of whole nodules

In some preliminary experiments conducted to determine the standard procedure to be followed in later work it was observed that sometimes glucose, particularly at concentrations of 5 per cent, slightly lowered the Q_{O_2} and increased the R.Q. values. In table 3

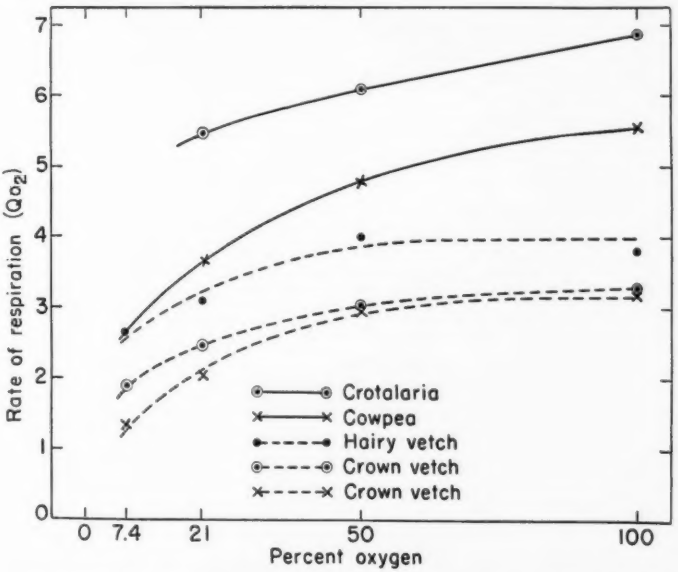

Fig. 5.—Effect of partial pressure of oxygen on rate of respiration of crushed nodules

are given data on the sugar effect at different partial pressures of oxygen. A portion of these results is plotted in figure 6.

In the experiments conducted in air, where the oxygen supply was inadequate for maximum rate of respiration, the R.Q. values were markedly increased by sugar, even at a concentration of 1 per cent, and still more so at higher percentages. The effect of the highest sugar concentration upon the Q_{O_2} values was usually not marked, although in a few cases they were lowered slightly. When 80 or 100 per cent O_2 was substituted for air, however, the R.Q. values, even with 5 per cent glucose added, remained near 1.0 and the Q_{O_2} values were not significantly depressed.

These observations constitute further evidence for the view that the supply of oxygen inside the nodule maintained in air is limited. Under these conditions of deficient oxygen the addition of glucose in relatively high concentrations has the effect of increasing somewhat

TABLE 3

EFFECT OF GLUCOSE CONCENTRATION UPON THE RATE OF RESPIRATION AND RESPIRATORY QUOTIENTS AT DIFFERENT PARTIAL PRESSURES OF OXYGEN

EXPERIMENT NO.	PLANT SPECIES	SIZE OF NODULES	GLUCOSE (%)	O_2 IN N_2 (%)	$\acute{Q}_{O_2}$	Q_{O_2}	$Q_{O_2}(N)$	R.Q.
28.....	Lespedeza	Medium	0	21	0.281	1.69	22.8	1.17
28.....	Lespedeza	Medium	1	21	0.300	1.80	24.3	1.37
28.....	Lespedeza	Medium	2.5	21	0.242	1.46	19.6	2.10
28.....	Lespedeza	Medium	5	21	0.202	1.21	16.3	2.43
29.....	Lespedeza	Medium	0	80	0.80	4.80	64.6	0.99
29.....	Lespedeza	Medium	1	80	0.85	5.11	68.8	1.03
29.....	Lespedeza	Medium	2.5	80	0.78	4.67	62.9	1.07
29.....	Lespedeza	Medium	5	80	0.84	5.06	68.2	1.11
30.....	Lespedeza	Medium	0	21	0.27	1.58	19.8	1.11
30.....	Lespedeza	Medium	1	21	0.29	1.72	21.6	1.40
30.....	Lespedeza	Medium	5	21	0.07	0.43	5.4	7.68*
30.....	Lespedeza	Medium	0	80	0.76	4.48	56.2	0.97
30.....	Lespedeza	Medium	1	80	0.88	5.13	64.3	1.03
30.....	Lespedeza	Medium	5	80	0.74	4.36	54.7	1.07
30.....	Lespedeza	Medium	0	100	0.86	5.02	62.8	0.94
30.....	Lespedeza	Medium	1	100	0.93	5.45	68.4	1.01
30.....	Lespedeza	Medium	5	100	0.86	5.04	63.2	1.07
81.....	Lespedeza	Small	0	21	0.229	1.24	17.6	0.98
81.....	Lespedeza	Small	1	21	0.238	1.30	18.3	1.29
81.....	Lespedeza	Small	2	21	0.262	1.43	20.2	1.38
81.....	Lespedeza	Small	3	21	0.247	1.35	19.0	1.49
81.....	Lespedeza	Small	4	21	0.196	1.07	15.1	1.53
81.....	Lespedeza	Small	5	21	0.248	1.35	19.1	1.63
87.....	Hairy vetch	Small	0	21	0.57	3.83	57.7	1.15
87.....	Hairy vetch	Small	1	21	0.58	3.85	58.0	1.44
87.....	Hairy vetch	Small	2	21	0.55	3.66	55.2	1.55
87.....	Hairy vetch	Small	3	21	0.57	3.79	57.1	1.54
87.....	Hairy vetch	Small	4	21	0.53	3.59	54.1	1.83
87.....	Hairy vetch	Small	5	21	0.53	3.53	53.1	1.66

* This figure is the result of one determination only and is undoubtedly incorrect.

the evolution of CO_2. The explanation for this probably involves a number of factors, but in the light of other data to be published later, it is highly probable that the abundant sugar supply favors a partially anaerobic breakdown of sugar inside the nodule. The outside layers of the nodule would presumably continue to use the available oxygen supply for normal aerobic oxidation of sugar, and

possibly of some of the intermediate products of anaerobic metabolism generated in the center of nodules. The net result of these two processes would of course be an increase in the R.Q., since the fermentative step would result in the formation of intermediate products and the evolution of CO_2 with limited or no oxygen uptake.

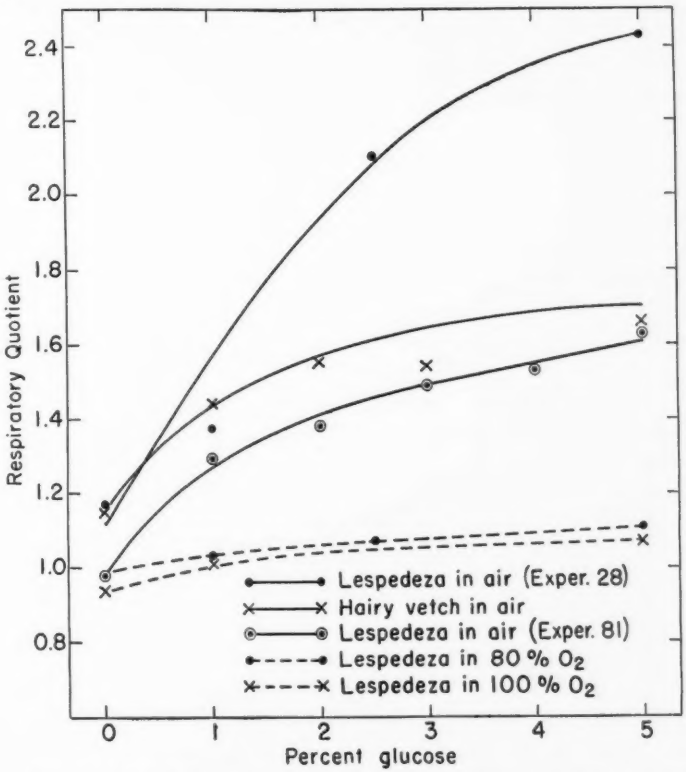

FIG. 6.—Effect of glucose concentration on respiratory quotient of nodules maintained in different partial pressures of oxygen.

Discussion

Although these studies show that most detached nodules maintained in air in Warburg vessels have a limited internal oxygen supply, it should be borne in mind that such methods of experimentation necessarily represent artificial conditions. When attached

to the plant, the movement of dissolved oxygen into, and of CO_2 from, the center of the nodule through the vascular strands may possibly be a significant factor. The direct diffusion of oxygen through the walls of the nodule under natural conditions may also be more rapid. In the experiments the nodules were constantly shaken in a thin layer of the basal medium (2 cc. per 100–200 mg. nodules), whereas in nature they are of course comparatively dry. In a few determinations on the rate of respiration of nodules kept in Warburg vessels without basal medium, the Q_{O_2} values were slightly lower than when the usual quantity of liquid medium was added. This indicates that oxygen penetration through the cortex of nodules under experimental conditions was as rapid as under field conditions. The penetration may well have been even more rapid, since in soils, particularly the heavier ones, air movement is retarded and in some cases the partial pressure of O_2 in soil air may be appreciably less than in normal air.

While the facts indicate that under field conditions the oxygen supply within the center of most nodules, and particularly of the larger ones, is limited, it cannot be inferred that a strict anaerobic or fermentative metabolism necessarily occurs. As already pointed out, an R.Q. characteristic of aerobic glucose utilization is frequently obtained under conditions where the oxygen supply is inadequate for maximum Q_{O_2} values. This indicates that true fermentation does not occur to any appreciable extent until the oxygen supply is rather limiting, or else if a fermentative step does occur the intermediate products are later oxidized to CO_2 and water.

Since nitrogen fixation occurs in nodules in air, where the O_2 concentration is not over 21 per cent and may be somewhat less, and commonly in nodules clearly larger than the largest which would give an R.Q. in air as low as unity, the conclusion seems evident that it occurs normally under partial anaerobiosis. These considerations constitute in no sense a denial of the often observed importance of soil aeration to legumes, but merely call attention to the condition within the nodule, where fixation occurs.

Summary

Respiration studies were undertaken with a view to obtaining fundamental information concerning the metabolic processes of

legume nodules, inasmuch as the knowledge gained should facilitate investigation of the process of nitrogen fixation. The results obtained by standard manometric procedures with detached nodules from eight species of legumes may be summarized as follows:

1. The rates of respiration of nodules maintained in various oxygen-nitrogen mixtures increased as the oxygen percentage increased. The Q_{O_2} values varied between 0.62 and 6.25 with an average of about 2.2 in air, and were about 2.4 times as great in pure oxygen, with maxima for the eight species varying between 4.3 for soybean and 8.9 for hairy vetch.

2. The respiratory quotients increased with increase in size of nodule and with decrease in concentration of oxygen. In air the values varied between 1.0 and 2.0, depending largely on the size of the nodules. In pure oxygen, excluding very large nodules, the values averaged 1.03.

3. The addition of glucose to the nutrient medium produced a mean increase in Q_{O_2} of about 12 per cent in air and 14 per cent in 100 per cent oxygen. It increased the R.Q. at the lower concentrations of oxygen but not appreciably in pure oxygen.

4. These results with detached nodules are interpreted as indicating that with most nodules maintained in air, or with large nodules at oxygen concentrations even as high as 100 per cent, the interior of the nodules is under anaerobic or partially anaerobic conditions. The evidence further indicates that under natural conditions in the soil the oxygen supply inside most nodules is also limited.

5. Crushed nodules gave higher Q_{O_2} values when maintained in air than did whole nodules, but usually lower values than whole nodules in pure oxygen. This method of increasing the aeration of the inner cells of the nodules was not very satisfactory.

6. The nodules of all the species showed rather similar responses, the variations between species being not much greater than that within species.

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BIOCHEMICAL NITROGEN FIXATION STUDIES

II. COMPARATIVE RESPIRATION OF NODULES AND ROOTS, INCLUDING NON-LEGUME ROOTS

FRANKLIN E. ALLISON, C. A. LUDWIG, F. W. MINOR AND SAM R. HOOVER[1]

(WITH TWO FIGURES)

Introduction

In the preceding paper (3) of this series data were presented showing the rates of respiration and respiratory quotients of various species and sizes of detached legume nodules maintained in various partial pressures of oxygen in nitrogen. These studies showed Q_{O_2} values averaging about 2.2 in air and 5.4 in pure oxygen. The R.Q. values were near 1.0 for nodules maintained in pure oxygen but frequently considerably higher than this when kept in air. It was concluded that the supply of oxygen inside most nodules maintained in air is limited.

The purpose of the present study was to determine to what extent nodule metabolism differs from root metabolism, particularly that of legume roots. Since nodules are filled with bacteria there seems to be a rather widespread belief that their metabolism, as evidenced by rate of respiration, is markedly higher than that of root tissues.

Methods

The methods used were essentially as described in the preceding paper and consisted for the most part of the standard manometric procedures (4).

In addition to the legumes listed previously (3), a limited amount of work was done with white clover (*Trifolium repens* L.) and lima bean (*Phaseolus lunatus* L.). The non-legume species studied included corn (*Zea mays* L.), oat (*Avena sativa* L.), potato (*Solanum*

[1] The writers are indebted to ELLEN K. RIST of this laboratory for the nitrogen analyses and to E. C. BUTTERFIELD of the Bureau of Plant Industry for supplying much of the plant material.

tuberosum L.), tomato (*Lycopersicon esculentum* Mill.), cabbage (*Brassica oleracea* L.), crabgrass (*Digitaria sanguinalis* (L.) Scop.), pigweed (*Amaranthus retroflexus* L.), narrow leaved plantain (*Plantago lanceolata* L.), broad leaved plantain (*P. rugelii* Dcne.), ragweed (*Ambrosia artemisiaefolia* L.), cocklebur (*Xanthium* sp.), and jimson weed (*Datura* sp.).

The nodules were obtained from plants in an active vegetative stage and were prepared for use as described in the preceding paper. When comparative studies of the respiration of nodules and legume roots were made, the root samples were, with few exceptions, taken from the same batch of plants as were the nodules. The non-legume roots were obtained from young plants of approximately similar age and condition, and usually from plants grown on the same type of soil as the legumes. The same procedure was followed in preparing them for use as in the case of the nodules. They were washed thoroughly, most of the adherent water removed by placing between filter papers, and cut into lengths of 0.5–1 cm. to permit more uniform sampling and easy introduction into the small respiration vessels. After bringing the plants into the laboratory they were kept in ice water or in a refrigerator at about 5° C. for as much as possible of the time that necessarily elapsed before starting the experiment. The studies with both roots and nodules were carried out with unsterilized material. Most of the Q_{O_2} and R.Q. results are based upon periods of 2–6 hours (usually 3–5 hours), during which time bacterial action was not appreciable. The usual size of the nodules and root samples was 150–200 mg. moist weight in 2 cc. of the usual basal medium (3), containing minerals but no nitrogen, and with or without glucose as desired.

In the frequent references to the comparative rates of respiration of small nodules and small roots which follow it is not meant to imply that the two types of plant material are of like diameter. Actually the small nodules had a diameter of about 0.5–2.5 mm. whereas the small roots seldom exceeded 1 mm. in diameter and a considerable portion was near 0.1 mm. This means, of course, that when maintained in air the small roots were much better supplied with oxygen than were the small nodules.

Experimental results

In reporting and interpreting the results, the plan used previously (3) of grouping all the pertinent data from a large number of experiments that deal with a given variable will be followed. In many of the earlier experiments, dealing primarily with nodule respiration, it was common to use roots in one or two of the respiration vessels as a control upon the nodule results, as well as to obtain a general idea of how nearly they behave like nodules. In later work many experiments dealt exclusively with roots. Rates of respiration, as in the preceding paper, are reported in terms of ml. O_2 consumed per mg. dry weight, Q_{O_2}; per mg. moist weight, $\acute{Q}_{O_2}$; and per mg. of tissue nitrogen, $Q_{O_2}(N)$. The figures as given are with few exceptions the averages of two or more determinations.

Rates of respiration and respiratory quotients of nodules and legume roots maintained in various partial pressures of oxygen in nitrogen

In table 1 are shown the Q_{O_2} values for the roots of several species of legumes maintained in nutrient solutions in various partial pressures of oxygen in nitrogen. A few values for nodules, when direct comparisons were made, are also included. A portion of these data are plotted in figure 1. Fairly typical time curves (experiment no. 68) showing the course of respiration of roots and nodules with and without glucose are shown in figure 2. In correlating the root respiration results with the corresponding values for nodules, it is necessary to bear in mind not only the data on nodules reported in table 1 but also the results presented in the first paper of this series.

The Q_{O_2} values for roots in air (table 1) varied between 0.57 (for a sample of large roots) and 4.19, with an average of 2.1. There was no marked difference between the Q_{O_2} values for roots and for the few samples of nodules reported here. In the previous paper the average Q_{O_2} value reported for a large number of samples of nodules maintained in air was 2.2. The rate of oxygen consumption per milligram of undried tissue ($\acute{Q}_{O_2}$) was somewhat higher with nodules than with roots, however, because young succulent roots ordinarily have a higher moisture content. Discussion of the $Q_{O_2}(N)$ data will be given in a later section of this paper.

TABLE 1

COMPARATIVE RATES OF RESPIRATION AND RESPIRATORY QUOTIENTS OF LEGUME ROOTS AND NODULES MAINTAINED IN VARIOUS PARTIAL PRESSURES OF OXYGEN IN NITROGEN

Experiment no.	Plant species	Plant part	Size	Glucose (%)	O_2 in N_2 (%)	$\acute{Q}_{O_2}$	Q_{O_2}	$Q_{O_2}(N)$	R.Q.
8.....	Crown vetch	Nodules	Medium	3	21	0.213	.....	.....	.....
8.....	Crown vetch	Roots	Medium	3	21	0.207	.....	.....	.....
14.....	Crown vetch	Nodules	Medium	0	21	0.253	.....	.....	0.97
14.....	Crown vetch	Roots	Medium	0	21	0.271	.....	.....	1.08
14.....	Crown vetch	Roots	Medium	1	21	0.330	.....	.....	1.10
73.....	Crown vetch	Roots	Small	0	50	0.269	1.63	69.2	.....
73.....	Crown vetch	Roots	Small	1	50	0.299	1.80	76.6	.....
65.....	Crotalaria	Nodules	Small	0	100	0.749	5.74	72.0	.....
65.....	Crotalaria	Nodules	Small	1	100	1.020	7.82	96.8	.....
65.....	Crotalaria	Roots	Small	0	100	0.276	2.60	125.0	.....
65.....	Crotalaria	Roots	Small	1	100	0.376	3.55	170.5	.....
65.....	Crotalaria	Roots	Large	0	100	0.196	1.29	100.8	.....
65.....	Crotalaria	Roots	Large	1	100	0.317	2.09	163.3	.....
66.....	Crotalaria	Nodules	Small	0	21	0.353	2.30	29.2	.....
66.....	Crotalaria	Roots	Small	0	21	0.223	2.10	93.1	.....
66.....	Crotalaria	Nodules	Small	0	50	0.602	3.91	49.8	.....
66.....	Crotalaria	Roots	Small	0	50	0.219	2.06	91.4	.....
66.....	Crotalaria	Nodules	Small	0	100	0.813	5.28	67.2	.....
66.....	Crotalaria	Roots	Small	0	100	0.215	2.02	89.6	.....
66.....	Crotalaria	Roots	Small	1	100	0.300	2.82	125.4	.....
67.....	Crotalaria	Nodules	Small	1	21	0.406	2.93	33.8	.....
67.....	Crotalaria	Roots	Small	1	21	0.244	2.44	125.2	.....
67.....	Crotalaria	Nodules	Small	1	50	0.711	5.13	59.3	.....
67.....	Crotalaria	Roots	Small	1	50	0.269	2.69	137.9	.....
67.....	Crotalaria	Nodules	Small	1	100	0.944	6.82	78.7	.....
67.....	Crotalaria	Roots	Small	1	100	0.269	2.69	137.9	.....
67.....	Crotalaria	Roots	Small	0	100	0.195	1.95	100.0	.....
68.....	Crotalaria	Roots	Small	1	100	0.252	2.54	116.5	.....
69.....	Sweet clover	Roots	Small	0	21	0.305	1.99	67.5	.....
69.....	Sweet clover	Roots	Small	1	21	0.405	2.65	90.0	.....
76.....	White clover	Roots	Small	0	21	0.251	1.84	88.9	.....
76.....	White clover	Roots	Small	1	21	0.296	2.17	104.8	.....
76.....	White clover	Roots	Small	1	100	0.309	2.27	109.7	.....
77.....	Lima bean	Roots	Small	0	21	0.225	2.61	94.1	.....
77.....	Lima bean	Roots	Small	1	21	0.306	3.53	127.5	.....
77.....	Lima bean	Roots	Small	1	100	0.509	5.88	212.4	.....
32.....	Lespedeza	Roots	Small	0	21	0.271	1.50	75.1	0.80
32.....	Lespedeza	Roots	Small	1	21	0.424	2.35	117.5	0.97
32.....	Lespedeza	Roots	Small	0	80	0.372	2.06	103.0	0.68
32.....	Lespedeza	Roots	Small	1	80	0.543	3.01	150.4	0.94
32.....	Lespedeza	Nodules	Medium	1	80	0.782	4.74	57.1	1.06
33.....	Lespedeza	Roots	Medium	0	21	0.300	1.64	88.1	0.85
33.....	Lespedeza	Roots	Medium	0	80	0.293	1.60	86.1	0.79
34.....	Lespedeza	Roots	Medium	0	21	0.331	1.51	80.8	.....
35.....	Lespedeza	Roots	Medium	0	21	0.282	1.49	81.4	0.94
35.....	Lespedeza	Roots	Medium	1	21	0.379	2.01	109.8	0.94
69.....	Lespedeza	Roots	Small	0	21	0.202	1.14	65.5	.....
69.....	Lespedeza	Roots	Small	1	21	0.340	1.92	110.3	.....

TABLE 1—*Continued*

EXPERIMENT NO.	PLANT SPECIES	PLANT PART	SIZE	GLUCOSE (%)	O_2 IN N_2 (%)	$\acute{Q}_{O_2}$	Q_{O_2}	$Q_{O_2}(N)$	R.Q.
21.....	Soybean	Nodules	Small	2	21	0.334	2.05	35.8	1.79
21.....	Soybean	Roots	Small	2	21	0.162	2.76	48.3	.....
22.....	Soybean	Nodules	Small	2	21	0.146	0.89	15.6	.....
22.....	Soybean	Roots	Small	2	21	0.131	2.50	48.4	.....
38.....	Soybean	Roots	Large	2	21	0.138	0.57	50.4	1.33
38.....	Soybean	Roots	Small	2	21	0.343	1.92	114.3	0.87
38.....	Soybean	Roots	Small	2	100	0.369	2.06	122.6	1.00
38.....	Soybean	Roots	Large	2	100	0.250	1.04	91.4	.....
38.....	Soybean	Nodules	Small	2	100	0.967	4.19	88.0	1.03
39.....	Soybean	Nodules	Large	2	100	0.501	1.98	45.1	.....
39.....	Soybean	Roots	Large	2	100	0.382	1.69	110.7	.....
1.....	Cowpea	Nodules	Medium	0	50	0.394	.....	.....	.....
1.....	Cowpea	Roots	.....	0	50	0.312	.....	.....	.....
1.....	Cowpea	Nodules	Medium	3	50	0.408	.....	.....	.....
1.....	Cowpea	Roots	.....	3	50	0.341	.....	.....	.....
2.....	Cowpea	Nodules	Medium	1	50	0.224	.....	.....	.....
2.....	Cowpea	Roots	.....	1	50	0.117	.....	.....	.....
3.....	Cowpea	Nodules	Medium	1	50	0.760	.....	.....	.....
3.....	Cowpea	Roots	.....	1	50	0.226	.....	.....	.....
6.....	Cowpea	Nodules	Medium	3	21	0.094	.....	.....	.....
6.....	Cowpea	Roots	.....	3	21	0.097	.....	.....	.....
7.....	Cowpea	Nodules	Small	3	21	0.205	.....	.....	.....
7.....	Cowpea	Roots	.....	3	21	0.192	.....	.....	.....
68.....	Cowpea	Nodules	Small	0	100	0.765	4.45	75.3	.....
68.....	Cowpea	Nodules	Small	1	100	0.787	4.58	77.6	.....
68.....	Cowpea	Roots	Small	0	100	0.362	3.35	122.7	.....
68.....	Cowpea	Roots	Small	1	100	0.529	4.90	179.3	.....
71.....	Cowpea	Nodules*	Small	1	21	0.733	3.64	61.1	.....
71.....	Cowpea	Roots	Small	1	21	0.455	4.19	197.8	.....
71.....	Cowpea	Nodules*	Small	1	50	0.964	4.80	80.3	.....
71.....	Cowpea	Roots	Small	1	50	0.587	5.34	255.3	.....
71.....	Cowpea	Nodules*	Small	1	100	1.116	5.55	93.2	.....
71.....	Cowpea	Roots	Small	1	100	0.683	6.21	297.1	.....
71.....	Cowpea	Nodules	Small	1	100	0.776	3.86	64.8	.....

* Nodules in these vessels were crushed.

An increase in oxygen pressure above that in air had, on the average, a much smaller effect upon the Q_{O_2} values of roots than of nodules. The average Q_{O_2} value for roots kept in pure oxygen was 2.88, an increase of only about 35 per cent over the mean value in air, whereas the corresponding increase in nodular respiration (3) was about 135 per cent. The explanation for the difference depends upon the fact that nodules ordinarily are capable of a higher maximum respiration rate than are roots, but under ordinary atmospheric conditions not enough oxygen for these higher rates can diffuse into

the nodules. If roots are used that have diameters as great as those of nodules and have very low Q_{O_2} values in air, then there is also a marked increase in rate of respiration with increase in oxygen pressure. The maximum Q_{O_2} values observed for such roots are of course seldom as high as for nodules. Sometimes even small roots which happen to be relatively impervious to oxygen also show a marked response to increased oxygen pressure. The results obtained with

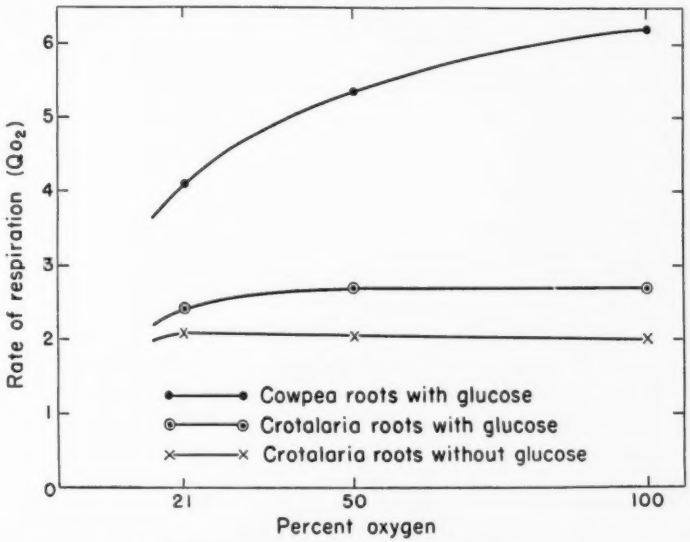

FIG. 1.—Effect of partial pressure of oxygen on rates of respiration of legume roots

small cowpea roots are an excellent example (fig. 1). As shown in the same figure, however, small crotalaria roots showed essentially no response to the additional oxygen.

A comparison of the rates of respiration of the roots of the various species of legumes shows no very marked differences for roots of comparable size, age, and condition. This is in agreement with the previous findings with regard to the Q_{O_2} values of nodules from various plant species.

The respiratory quotients of small roots maintained in nutrient solutions in air averaged 0.92 in the absence of added glucose and

0.97 when it was present. The values were therefore definitely lower than for nodules, owing primarily to the larger diameter and greater oxygen lack in the latter. Large roots also showed R.Q. values considerably higher than 1.0 but not so high as for the larger nodules.

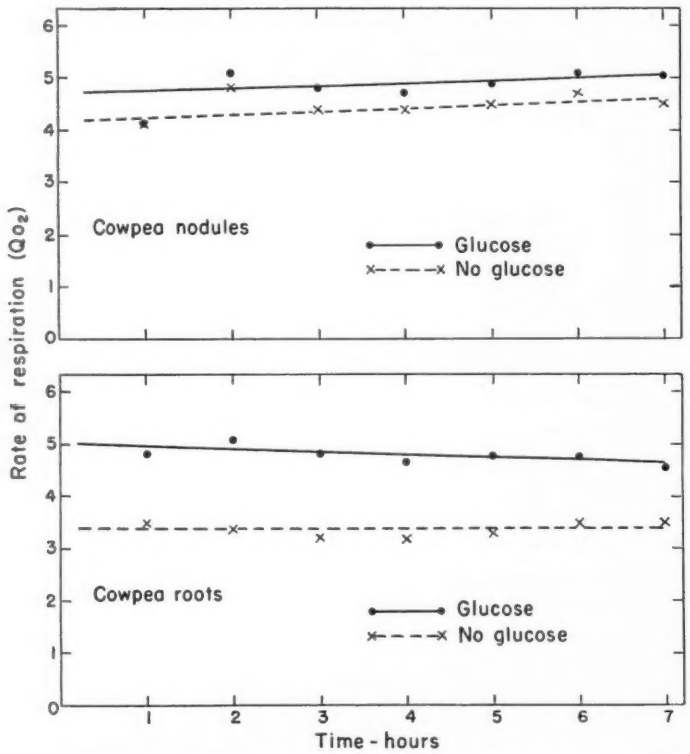

FIG. 2.—Rates of respiration of cowpea nodules and roots in 100 per cent oxygen in presence and absence of glucose.

Only a few R.Q. values were obtained with legume roots maintained in partial pressures of oxygen greater than in air, but these data show values somewhat lower than in air.

The data in table 1 indicate that legume roots are ordinarily more deficient in immediately available carbohydrates than are nodules.

As shown in table 1, the addition of glucose to roots increased the rate of respiration an average of about 40 per cent when direct comparisons were made. The beneficial effect of glucose was slightly greater in pure oxygen than in air. The increase with nodules due to glucose, as pointed out previously (**3**), was on the average about 12–14 per cent and was sometimes almost negligible. These observations are in harmony with the well known fact that young roots are essentially conducting vessels and relatively free from storage materials, whereas nodules are high in protein and not infrequently contain considerable starch.

A general summary of the results leads to the definite conclusion that young active nodules maintained in air usually respire at near the same rate per unit of dry matter as young succulent roots. But nodules have a capacity for respiring two or three times faster than roots if they are placed in pure oxygen so as to provide for more rapid penetration of the oxygen into the centers that are normally partially anaerobic. Of course this does not happen except under laboratory conditions, hence the Q_{O_2} values of young roots and nodules in nature are probably not greatly different.

Rates of respiration of legume and non-legume roots

The determination that legume nodules and root tissues behave much alike with regard to rates of respiration per unit dry weight under normal atmospheric conditions led to the inquiry as to whether there is any marked difference between legume and non-legume root tissues. There is need for quantitative data on this point, since there seems to be a rather general impression (**1**, p. 129), based upon limited quantitative data obtained in part from measurements made in the field, that legume roots respire considerably more rapidly than do non-legume roots. This view is not, however, universal. For instance, Smith (**6**) found that legumes produce no more CO_2 than do other crops. Table 2 gives data on this point for seven species of legumes and twelve of non-legumes.

The Q_{O_2} values for small legume roots maintained in air, including the values given in table 1, varied between 1.14 and 4.19 with an average of about 2.2; the corresponding values for the non-legumes varied between 1.03 and 3.67 with an average of 2.3. There was

TABLE 2

COMPARISON OF RATES OF RESPIRATION OF SMALL LEGUME AND NON-LEGUME ROOTS

EXPERIMENT NO.	PLANT SPECIES	GLUCOSE (%)	O_2 IN N_2 (%)	$\acute{Q}O_2$	QO_2	$QO_2(N)$	R.Q.
68	Cowpea	0	100	0.362	3.35	122.7	.....
68	Cowpea	1	100	0.529	4.90	179.3	.....
68	Corn	0	100	0.244	2.89	173.7	.....
68	Corn	1	100	0.319	3.78	227.4	.....
68	Crotalaria	1	100	0.252	2.54	116.5	.....
69	Sweet clover	0	21	0.305	1.99	67.5	.....
69	Sweet clover	1	21	0.405	2.65	90.0	.....
69	Lespedeza	0	21	0.202	1.14	65.5	.....
69	Lespedeza	1	21	0.340	1.92	110.3	.....
69	Pigweed	0	21	0.248	2.48	95.4	.....
69	Pigweed	1	21	0.367	3.67	141.2	.....
69	Tomato	0	21	0.204	1.92	101.6	.....
69	Tomato	1	21	0.276	2.61	138.1	.....
69	Oat	0	21	0.121	1.21	73.3	.....
69	Oat	1	21	0.166	1.66	100.6	.....
69	Crab grass	0	21	0.211	2.07	101.5	.....
69	Crab grass	1	21	0.336	3.29	161.3	.....
69	Narrow leaved plantain	1	21	0.142	1.03	143.0	.....
73	Crown vetch	0	50	0.269	1.63	69.2	.....
73	Crown vetch	1	50	0.299	1.80	76.6	.....
73	Ragweed	0	50	0.168	2.16	106.2	.....
73	Ragweed	1	50	0.211	2.70	133.0	.....
73	Broad leaved plantain	0	50	0.119	1.31	92.9	.....
73	Broad leaved plantain	1	50	0.196	2.16	153.2	.....
76	White clover	0	21	0.251	1.84	88.9	.....
76	White clover	1	21	0.296	2.17	104.8	.....
76	Cabbage	0	21	0.289	2.00	83.4	.....
76	Cabbage	1	21	0.370	2.55	106.3	.....
76	Potato	0	21	0.207	2.35	92.2	.....
76	Potato	1	21	0.299	3.40	133.3	.....
76	White clover	1	100	0.309	2.27	109.7	.....
76	Cabbage	1	100	0.387	2.67	111.3	.....
76	Potato	0	100	0.183	2.07	81.2	.....
76	Potato	1	100	0.303	3.44	134.9	.....
77	Lima bean	0	21	0.225	2.61	94.1	.....
77	Lima bean	1	21	0.306	3.53	127.5	.....
77	Cocklebur	0	21	0.137	1.98	85.9	.....
77	Cocklebur	1	21	0.232	3.34	145.9	.....
77	Jimson weed	0	21	0.130	1.84	69.6	.....
77	Jimson weed	1	21	0.252	3.55	134.5	.....
77	Lima bean	1	100	0.509	5.88	212.4	.....
77	Cocklebur	1	100	0.217	3.12	135.5	.....
77	Jimson weed	0	100	0.114	1.61	61.0	.....
77	Jimson weed	1	100	0.258	3.63	137.4	.....
53	Oat	0	100	0.206	1.75	125.0	0.92
53	Oat	1	100	0.242	2.05	146.4	0.96
54	Oat	0	100	0.261	1.94	137.3	0.92
54	Oat	1	100	0.295	2.19	155.1	0.90

TABLE 2—*Continued*

Experiment no.	Plant species	Glucose (%)	O_2 in N_2 (%)	$\dot{Q}O_2$	QO_2	$QO_2(N)$	R.Q.
72	Corn	0	21	0.123	1.35	75.0	0.97
72	Corn	1	21	0.162	1.77	98.3	1.04
72	Corn	0	100	0.121	1.32	73.7	0.88
72	Corn	1	100	0.158	1.73	95.8	0.91
72	Corn*	1	100	0.183	2.32	133.3	1.00
74	Corn	0	21	0.178	1.78	153.5	0.89
74	Corn	1	21	0.206	2.06	177.6	0.95
74	Corn	0	100	0.181	1.81	155.6	0.92
74	Corn	1	100	0.229	2.29	197.0	0.91

* Very young succulent roots.

obviously no significant difference between the rates of respiration of the small legume and small non-legume roots, although, as would be expected, there were some species differences in the case of both groups of plants. Not enough determinations were made, however, to make certain that the values obtained were truly characteristic of species and not merely differences in the particular root samples studied. On the whole the QO_2 values for the twelve plant species varied within a comparatively narrow range.

When pure oxygen was used, the QO_2 values for the non-legume roots (table 2) averaged 2.4, whereas the corresponding value for the legume roots (table 1) was 2.9. It is probable that the small increase in QO_2 usually produced by increased oxygen pressure in both legume and non-legume roots is due only to the better aeration of inside cells.

The respiratory quotients of corn and oat roots (table 2) show average values for results obtained in air and in 100 per cent oxygen of 0.92 for roots not receiving glucose and 0.95 for those supplied with 1 per cent glucose. These non-legume roots therefore responded much like legume roots. In both cases the R.Q. values were lower than for nodules. The frequent failure to obtain R.Q. values of 1.0 with both legume and non-legume roots suggests that in the absence of added sugars these roots may have been oxidizing appreciable quantities of materials other than sugars, such as protein or alcohol, which are characterized by a lower R.Q. than that of sugar.

R.Q. values of less than 1.0 obtained in the presence of abundant glucose may be attributed to the incomplete oxidation of glucose to form appreciable quantities of organic acids or other substances which would themselves on oxidation yield R.Q. values greater than 1.0.

The data of table 2 show that small non-legume roots, like small legume roots, are usually deficient in available energy supply. An average of all of the data in this table shows that the addition of glucose to non-legume roots produced an increase of about 40 per cent in Q_{O_2}. Such a low Q_{O_2} in the absence of added sugar, together with an R.Q. of less than 1.0, is strongly indicative of true endogenous respiration, that is, the oxidation of protein and cell constituents other than normal carbonaceous reserve compounds.

Comparative rates of respiration per unit of nitrogen of legume nodules, legume roots and non-legume roots

In tables 1 and 2 and in the preceding paper (3) are presented data showing the rates of respiration per unit of nitrogen, $Q_{O_2}(N)$, for various species of legume nodules, legume roots, and non-legume roots maintained under the various experimental conditions already discussed. In order to facilitate the discussion of the $Q_{O_2}(N)$ data, a summary was made of all of these values, together with some additional similar data not reported in either of these papers, where both Q_{O_2} and $Q_{O_2}(N)$ figures were available. The additional data included several values for medium and large sized nodules. The average values are given in table 3.

In considering these data it is well to point out that the original individual values from which the averages were calculated deal with a large number of variables, and that an equal number of determinations involving each variable was not conducted for each group of plants both in air and in pure oxygen. The average values, however, are based on a rather large number of individual values and are believed to be representative.

It has just been pointed out that in air the Q_{O_2} values for non-legume roots, legume roots, and small nodules were not markedly different but averaged near 2.2. The value for nodules, given in table

3, however, is lower than that for the root tissues, owing to inclusion of the results with some of the larger nodules in the averages. In contrast, the $Q_{O_2}(N)$ values for non-legume roots and legume roots are three to four times greater than the value for nodules; the difference would have been reduced somewhat if only small nodules had been used. The slightly lower value for legume roots in comparison with that for non-legume roots may be attributed to the fact that the

TABLE 3

COMPARATIVE AVERAGE Q_{O_2} AND $Q_{O_2}(N)$ VALUES FOR LEGUME NODULES LEGUME ROOTS, AND NON-LEGUME ROOTS MAINTAINED IN AIR AND PURE OXYGEN

MATERIAL	Q_{O_2}	RELATIVE VALUES	$Q_{O_2}(N)$	RELATIVE VALUES
	AIR			
Non-legume roots	2.28	(100)	114.8	(100)
Legume roots	2.12	93	92.8	81
Legume nodules	1.85	81	27.8	24
	OXYGEN (100%)			
Non-legume roots	2.39	105	134.2	117
Legume roots	2.88	126	139.7	122
Legume nodules	4.98	218	73.3	64

legume roots were frequently slightly larger than the non-legume roots.

In pure oxygen the Q_{O_2} of nodules was on the average nearly twice as great as that for roots. In contrast, the average $Q_{O_2}(N)$ value for nodules was only slightly more than half as large as for root tissues. On a unit nitrogen basis, therefore, root tissues are considerably more active even in pure oxygen than are nodule tissues and much more so under ordinary atmospheric conditions. It should be borne in mind, however, that in the previous paper (**3**) it was shown that although the rate of nodule respiration increases with oxygen pressure, the maximum rate was apparently (**3**, fig. 2) not reached even

with small nodules maintained in pure oxygen. In view of this finding, and the fact that the average values reported in table 3 included numerous determinations with medium or larger sized nodules, it seems evident that if nodule tissues could be given all the oxygen that they could consume, the difference between the $Q_{O_2}(N)$ values of nodule and root tissues would be appreciably less. Nevertheless there does seem to be a real difference between small roots and nodules in the rate of respiration per unit of nitrogen in the tissues. This fact might be interpreted as indicating that a greater portion of the nitrogen of the nodules is in storage or translocatory materials than is the case in small roots. It is reasonable to expect this situation, of course, since the nodule cells contain an abundance of bacteria, a large portion of which seem to be inactive, and are also constantly supplying the host plant with soluble forms of nitrogen.

Discussion

These experiments show that there is, on the average, little or no difference between the rates of respiration of small legume and small non-legume roots. Frequently roots of legumes do show higher rates of respiration than those of non-legumes, but the contrary is also true. Legume nodules ordinarily possess the capacity in the presence of pure oxygen to respire about twice as fast as a given weight of root tissue; but, because of the limited oxygen supply inside, their rate of respiration under ordinary atmospheric conditions is usually not much, if any, greater than that of roots. In the case of nodules having diameters greater than about 2 or 3 mm., the Q_{O_2} is commonly lower than for root tissues. These findings do not necessarily disagree with those respiration results obtained in field experiments that show higher rates of root respiration for legumes than for non-legumes per unit of field area. Legumes, especially perennials, commonly have larger root masses than non-legumes, especially the small grain crops frequently studied. Furthermore, soils evolve varying quantities of CO_2, and this fact tends to complicate the interpretation of data obtained in the field.

The facts presented here, together with the respiration data of the preceding paper, show that in this respect nodule behavior is in general similar to that of plant root cells and resting bacteria (or

both) and not to that of actively growing rhizobia. HOOVER and ALLISON (**5, 2**) have shown that very actively growing rhizobia have Q_{O_2} values of 30–40, whereas truly resting rhizobia have Q_{O_2} values of 6–8 when supplied with adequate sugar. In the absence of substrate the endogenous respiration of the bacteria may be near 1–3. In the experiments reported here a Q_{O_2} value of about 9 represented the maximum observed with nodules, and that rate occurred only in pure oxygen. The average Q_{O_2} value of 5 in pure oxygen is therefore near the value that might be expected on the assumption that the nodule consists of plant cells filled with resting bacteria. The lower Q_{O_2} value for nodules maintained in air is of practical interest, but in attempting to determine the condition of the bacteria in the nodules it is best to put more weight upon the pure oxygen results, since the low Q_{O_2} values in air are due largely to the failure of oxygen to penetrate sufficiently rapidly to meet their needs. Hence such low values do not necessarily indicate bacteria incapable of normal activity. The conception of the nodule as a place where large amounts of carbohydrates are oxidized per unit of tissue is therefore incorrect. Instead, the data are in harmony with the idea, borne out by numerous morphological studies, that the nodule consists of plant cells filled largely with bacteroids that are comparatively inactive, particularly under ordinary atmospheric conditions. But the data do not definitely exclude the possibility that a considerable portion (10 per cent or more) of the bacteria may be growing fairly rapidly, since even such active bacteria may have comparatively low Q_{O_2} values (15–25).

In a previous paper from this laboratory, ALLISON (**1**) attempted, on the basis of the meager respiration data then available, to estimate the carbohydrate consumption in the nodule. On the assumption that legumes respire twice as rapidly as non-legumes, owing to the bacteria in the nodules, it was calculated that "the bacterial requirements for respiration and nitrogen fixation are probably not greater than 3 to 6 per cent of the total carbohydrate photosynthesized under good growing conditions." It was further stated that this estimate was believed to be high and that the host itself consumes most of the carbohydrate. On the basis of the present data showing that under natural field conditions legume nodules with

their bacteria respire at nearly the same rate as legume and non-legume roots, and not twice as fast, we can say definitely that the carbohydrate oxidized by the bacteria in nodules is indeed a small portion of the total photosynthesized. The bacterial respiration in the nodule is not much greater than that of the equivalent weight of the host plant tissues. These findings are in harmony with practical observations to the effect that inoculated legumes, even under inadequate light conditions, do not appear to suffer much, if any, more from insufficient carbohydrate than do uninoculated legumes supplied fixed nitrogen.

Summary

The results of respiration studies with detached nodules, legume roots, and non-legume roots from several species of plants, carried out according to standard manometric procedures, are as follows:

1. Rates of respiration of legume nodules and small legume and non-legume roots maintained in a nutrient solution in air averaged nearly the same, the Q_{O_2} values being about 2.2. Rates of respiration of the roots of various species of plants showed no marked differences for roots of comparable age, size, and condition.

2. Respiratory quotients of small legume and non-legume roots maintained in air were usually slightly less than 1.0 and hence were definitely lower than for nodules, although large roots may also show values considerably above 1.0 because of slow oxygen penetration.

3. Rates of respiration per unit of dry matter of legume nodules in pure oxygen were more than twice as great as in air, whereas values for the small legume and non-legume roots were usually not markedly affected by the additional oxygen. Nodules therefore have an inherent capacity for respiring at a more rapid rate than root tissues, provided the inner cells can get the necessary oxygen.

4. Small legume and non-legume roots are more deficient in available energy supply than are nodules, a mean increase in Q_{O_2} of about 40 per cent due to glucose additions to roots being obtained, compared with 12–14 per cent with nodules.

5. Rates of respiration per unit of nitrogen of legume and non-legume roots maintained in air were three to four times as great as for nodules. Even in pure oxygen the $Q_{O_2}(N)$ values of roots were on the average about twice as great as for nodules.

6. These data are in harmony with the idea, so frequently suggested by morphological studies, that the nodule consists of plant cells largely filled with comparatively inactive bacteria. The bacteria oxidize only a very small portion of the total carbohydrate photosynthesized by the host plant.

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CYTOLOGICAL STUDIES OF LILIUM TIGRINUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **509**

JONATHAN J. WESTFALL

(WITH SIXTY-NINE FIGURES)

Introduction

Because it is the only species of *Lilium* reported to possess both diploid and triploid forms, *L. tigrinum* Ker-Gawl. is of special interest. Early investigators reported only the diploid form, but descriptions and figures given in several papers indicate that some of the plants reported as diploids were in reality triploids.

COULTER, CHAMBERLAIN, and SCHAFFNER (**13**), BELLING (**8**), and SIANG (**36**) reported 12 pairs of synaptic mates in the meiotic divisions of the microsporocytes. More recently TAKENAKA and NAGAMATSU (**42**) reported that this species possessed 36 somatic chromosomes. SATO (**32**) confirmed this work on the single-flowered variety, and reported that *L. tigrinum* var. *flore-pleno* also has 36 chromosomes. TAKENAKA (**41**) observed lagging, fragmentation, and other irregularities in the meiotic divisions of the microsporocytes of *L. tigrinum*. In the following year SASS (**30**) found similar irregularities. MATHER (**21**) extended the studies to include the additional *tigrinum* varieties *Fortunae giganteum* and *splendens*. Both were found to possess 36 chromosomes. CHANDLER, PORTERFIELD, and STOUT (**11**) studied chromosome morphology and microsporogenesis in both the diploid and triploid forms. The existence of this self-fertile species of *Lilium*, which proved to be of the diploid type, had previously been reported by PRESTON (**25**). Chromosome counts of a number of seedlings of the triploid *L. tigrinum* were reported by SATO (**33**).

Following the account of development of the megagametophyte of *Fritillaria persica*, in which BAMBACIONI (**1**) described the process wherein four nuclear divisions intervene between the archesporial cell and the egg instead of three, as was formerly supposed, other members of the Liliaceae were examined or reexamined in rapid succession.

BAMBACIONI and GIOMBINI (2) reported a similar process in *Tulipa gesneriana*. This was followed by the work of BAMBACIONI-MAZETTI (3) on *Lilium bulbiferum*, *L. candidum*, and *Tulipa praecox*. Both *T. praecox* and *T. gesneriana* have since been found to be triploids (15). Although her report of the development of the megagametophyte of these two species emphasizes the division and orientation of the nuclei rather than the determination of the exact numbers of chromosomes, some of the illustrative figures included show lagging chromosomes and micronuclei similar to those reported for other triploid plants.

COOPER (12) described the method of development of the megagametophyte in *Lilium henryii* and examined preparations of *L. speciosum*, *L. philadelphicum*, *L. longiflorum* var. *eximium*, *L. regale*, *L. auratum* var. *platyphyllum*, *L. elegans*, *L. martagon*, *L. pardalinum*, and *L. philippinense*. Later SANTOS (29) described the complete process in *L. philippinense*. In all species of *Lilium*, *Fritillaria*, and *Tulipa* examined it was found that in the course of the third nuclear division three of the four nuclei formed as the result of the second meiotic division migrate to the chalazal end of the megagametophyte, and there the three spindles fuse and undergo mitosis. At the same time the lone nucleus at the micropylar end also divides mitotically, so that a second 4-nucleate stage results. Instead of all nuclei being haploid, however, as in the first 4-nucleate stage, the two micropylar nuclei are at the later stage haploid, but the two chalazal nuclei are triploid. One further mitotic nuclear division occurs, resulting in the formation of four nuclei at each end of the megagametophyte. One nucleus from each end, the polar nuclei, move toward the center and unite, the nucleus from the chalazal end contributing $3n$ chromosomes and the nucleus from the micropylar end bringing the total count of the fusion nucleus to $4n$ in the diploid plants. In the process of fertilization one of the male gametes unites with the egg at the micropylar end to form the zygote, while the other unites with the polar nucleus to form the primary endosperm nucleus. This type of megagametophyte development has been designated by SCHNARF (35) as the "*Fritillaria* type," to avoid confusion with the "*Lilium* type" in the older sense.

In other members of the Liliaceae microsporogenesis has been

studied in the triploid forms of *Hyacinthus* by BELLING (6); *Hosta* by YASUI (45); *Hemerocallis* by BELLING (7); *Tulipa* by DARLINGTON (14), NEWTON (24), and WOODS (44); and *Allium* by LEVAN (20).

Among the limited number of reports on the development of the megagametophyte in triploids are those of MORINAGA and FUKUSHIMA (22) on *Oryza* and of SATINA and BLAKESLEE (31) on *Datura stramonium*.

If assortment of chromosomes and distribution of nuclei were the same in the development of the female gametophyte as in the male, studies of both would not be necessary; but the chromosome numbers of the progeny of triploids may be different, depending on whether the triploid is the pollen parent or the seed parent or both. As a general rule extra chromosomes are more often transmitted to the progeny by the female gametes than by the male (14), possibly because of complete failure of growth of the abnormal pollen grains or their failure to compete with pollen grains having the haploid set of chromosomes. Other features, as chromosome lagging, fragmentation, and random assortment—characteristic of autotriploids—as well as the type of division and arrangement of nuclei, make *Lilium tigrinum* particularly interesting for cytological study.

Since SCHAFFNER (34), SASS (30), TAKENAKA (41), and CHANDLER, PORTERFIELD, and STOUT (11) previously reported studies of microsporogenesis in *L. tigrinum*, the present study devotes attention to megasporogenesis and development of the megagametophyte, but makes frequent reference to observations on microsporogenesis.

Material and methods

Anthers and ovaries of *L. tigrinum* var. *splendens* were gathered from plants grown in the experimental garden at the University of Chicago and at Wychwood on Lake Geneva, Wisconsin, during late July and the first two weeks in August of the summer of 1938. The bulbs were originally obtained from Wayside Gardens, Mentor, Ohio.

The iron-aceto-carmine smear method was used in making preliminary studies and in determining the proper stages for fixation of the anthers. In most instances the first meiotic division was found to take place when the buds were about $1\frac{1}{4}$ inches in length, or

about 2–3 days before anthesis. Anther material was fixed at different times during the day over a period of 3 weeks. Ovaries were fixed one day before anthesis and continued at 24-hour intervals until 120 hours after anthesis. The ovaries were cut either longitudinally or transversely to reduce the distance the fixatives had to penetrate. Some of the ovules were dissected out before being fixed.

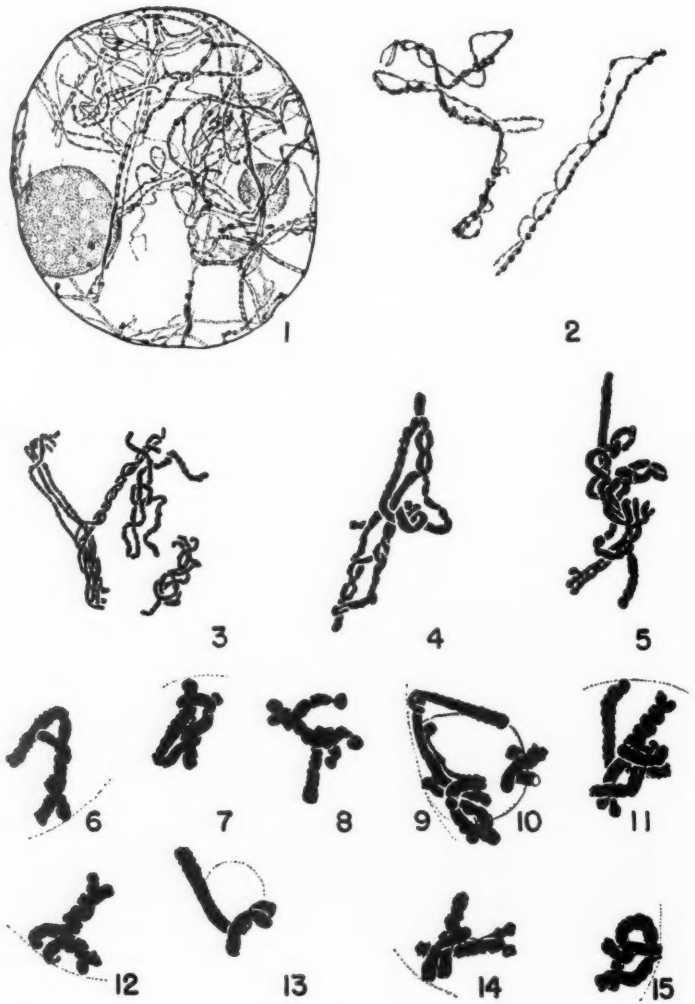
Smears of the anthers were fixed in Belling's modification of Navashin's and in Carnoy's solutions. Other anthers and the ovaries were allowed to remain in Carnoy's for about 80 seconds, after which they were placed in Navashin's solution. This treatment, as well as using Flemming's or Navashin's alone, gave good fixations. The butyl-alcohol paraffin method of imbedding was used, and sections were cut chiefly at 25–30 μ, although some sections for critical studies of the chromosomes were cut as thin as 6 μ. Either Heidenhain's iron-alum haematoxylin or the crystal violet-iodine method was used in staining all paraffin sections.

Observations were made with either a 10× or a 15× compensating ocular and a 100× fluorite objective. Drawings were made at table level with a camera lucida.

Observations

Critical examination of stages in the development of *L. tigrinum* var. *splendens* indicates that with few exceptions development follows the general plan reported for other species of *Lilium*, *Fritillaria*, and *Tulipa*. In the present paper no attempt is made to reiterate the story of division and orientation of nuclei or to illustrate all stages in developmental sequence. Only those phases in which *L. tigrinum* has been found to differ from other reported species are given particular attention.

In the early prophases of both the megasporocytes and the microsporocytes, chromosomes occur as single or as paired threads. Pairing, first evident at zygotene, is even more apparent at pachytene, when the threads have increased in chromaticity and have contracted somewhat longitudinally. At this stage (fig. 1) two of the synapsed chromosomes are closely associated and coiled throughout long segments, while the third apparently homologous chromosome



FIGS. 1–15.—Prophases and diakinesis in megasporocytes: fig. 1, nucleus at pachytene; fig. 2, late pachytene threads showing type of chromosome association in detail; figs. 3–5, diplotene and early diakinesis showing entangled trivalent groups; figs. 6–15, trivalent associations of single nucleus at diakinesis.

is in contact with the two at irregular intervals (fig. 2). In most instances the two closely synapsed chromosomes appear to have contracted longitudinally more rapidly after pairing than did the third member. Contraction does not seem to be dependent on pairing but is initiated sooner and progresses more rapidly when pairing has taken place. Differential contraction in some instances causes apparent failure of correspondence in chromomere arrangement in homologous chromosomes. At diplotene and early diakinesis (figs. 3–5) the chromatids of each chromosome are plainly visible, and frequently the members of two or more trivalent associations are

TABLE 1

FREQUENCY OF TRIVALENT, BIVALENT, AND UNIVALENT CHROMOSOME ASSOCIATIONS AT DIAKINESIS AND METAPHASE I; DATA FOR 500 MICROSPOROCYTES

ASSOCIATIONS	FREQUENCY	PERCENTAGE FREQUENCY
12 trivalents	293	58.6
11 trivalents, 1 bivalent, and 1 univalent	155	31.0
10 trivalents, 2 bivalents, and 2 univalents	39	7.6
9 trivalents, 3 bivalents, and 3 univalents	11	2.2
8 trivalents, 4 bivalents, and 4 univalents	2	0.6
Less than 8 trivalents	0	0.0
Mean no. of trivalents for entire sample		11.45

entangled so as to produce an apparent secondary association. This is interpreted as a result of chance arrangement of chromosomes preceding pairing, so that true or false interlocking results, rather than as interchange of parts between nonhomologous chromosomes. At diakinesis a single nucleus generally contains three nucleoli of different sizes, although more or less than this number may be present.

Preliminary studies of chromosome associations in the microsporocytes and megasporocytes at diakinesis show no significant differences in the degree of frequency of trivalents, bivalents, and univalents in the two types of cells. Since it is easier to make counts from smear preparations of the microsporocytes, they were used in determining the frequency of different types of associations.

Chromosomes of two or more trivalent groups often remain inter-

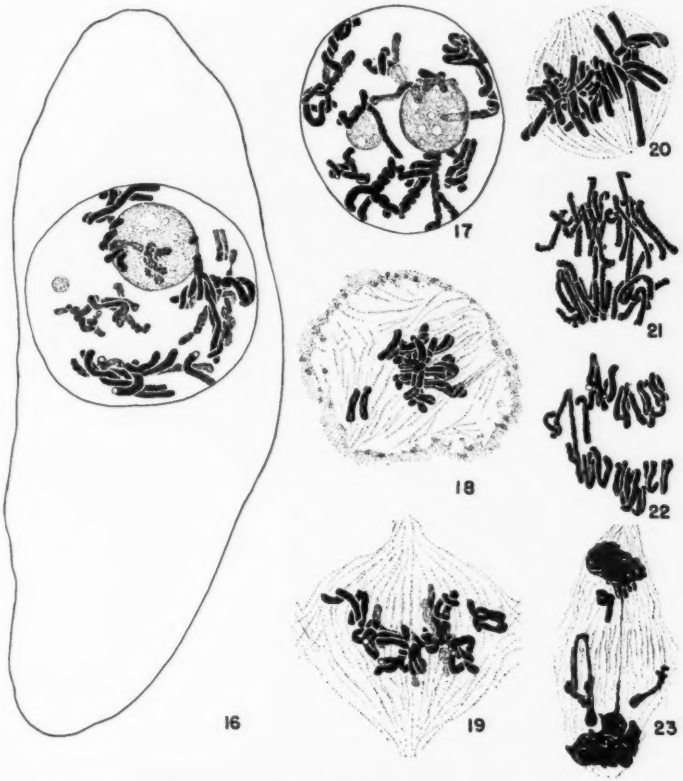
meshed throughout diakinesis (figs. 9, 11), but 12 separate trivalent associations occur more frequently.

Trivalents shown in figures 5–15 were drawn from a single nucleus, and in all cases at least one chromosome of each association was in contact with the nuclear membrane. The trivalents are oriented in such a manner that each association, with the exception of those entangled, is well separated from the next, as if repelled. This is not so apparent in single-plane drawings as it is in the material from which they were drawn. In figure 16 the chromosomes are grouped as 11 trivalents, 1 bivalent, and 1 univalent. All chromosomes are grouped as 12 trivalent associations in figure 17. Two univalents are shown apart from the compactly grouped trivalent and bivalent mass in figure 18. Dissociation of some of the trivalents is shown at early metaphase in figure 19.

First meiotic division

The arrangement of chromosomes at the equatorial plane during metaphase of the first meiotic division seems to be without unusual features (figs. 19, 20). Only occasionally is a univalent left outside the plane toward the poles. The first meiotic division is generally characterized by complete disjunction of all members of trivalent and bivalent associations, although there are two notable exceptions to this general rule. First, chromatids occasionally fail to disjoin at anaphase and tend to adhere near their ends, which are bent out at right angles to the axes of the two chromatids. This type of behavior is shown in the center of figure 21 and in the first of the three bridges of figure 53. The chromatids may eventually disjoin, or nondisjunction may persist in the microsporocytes until after the 2-celled stage. The plainly visible ends of the bridging chromatids and the absence of acentric fragments distinguish this from a more common type of bridge shown to the right of center in figure 21 and to the left of center in figure 22. In figure 23, in addition to four lagging chromosomes between the two organizing telophase nuclei, there is a fragment and an attenuated chromatid bridge which appears to be stretched almost to the breaking point. The frequency of such bridges appears to be about the same in microsporogenesis and megasporogenesis. Of 603 microsporocytes examined, seventy,

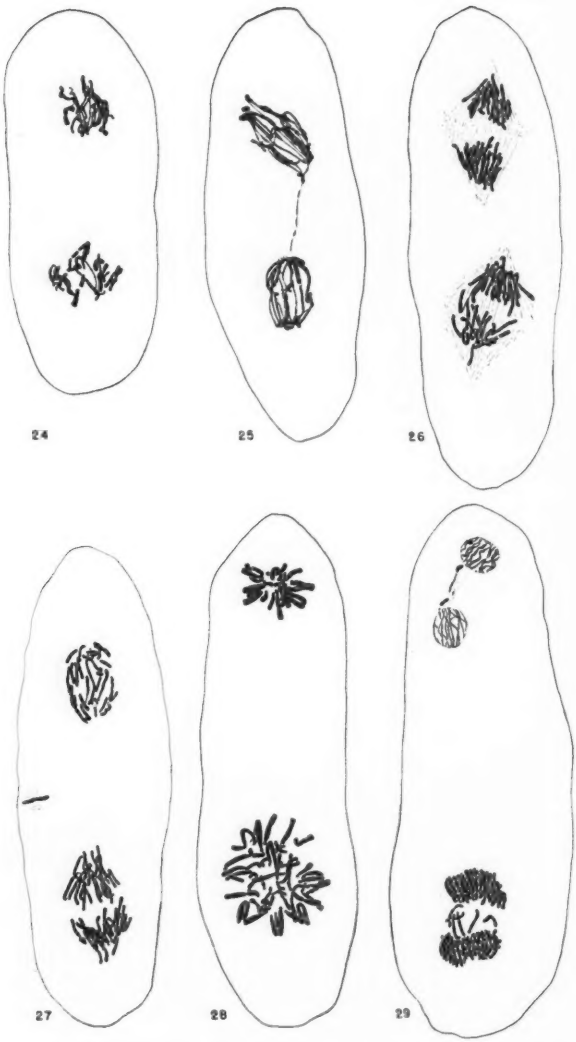
or 11.6 per cent, showed the presence of anaphase chromatid bridges. These bridges occasionally persist even after the formation of the



FIGS. 16–23.—Fig. 16, megasporocyte at diakinesis showing 11 trivalents, 11 bivalents, and 1 univalent; fig. 17, nucleus at diakinesis showing trivalent associations and nucleolar attachments; figs. 18–20, metaphase of first meiotic division; figs. 21, 22, anaphase showing chromatid bridges; fig. 23, telophase showing lagging chromosomes and attenuated chromatid bridge.

division figure of the second meiotic division (fig. 25). In figure 30 the two spindles of the second meiotic division are seen unusually close together, still joined by a persisting chromatid connection.

The first meiotic division of the microsporocyte differs from that



FIGS. 24–29.—Fig. 24, metaphase of second meiotic division; fig. 25, early anaphase figures of second meiotic division; figs. 26, 27, anaphase of second meiotic division; fig. 28, metaphase of third nuclear division; fig. 29, 4-nucleate stage following third nuclear division, showing lagging at both micropylar and chalazal ends.

of the megasporocyte in that in the former a cell wall is formed between the two nuclei before the second division. As this cell wall forms it may cut in two any bridges persisting after the first division. More often, however, the bridge prevents the formation of the cell wall at the point through which it passes in connecting the two nuclei. Prevention of cell wall formation is regarded not so much as an inhibitive action on the part of the chromatin material as a mere replacement of the wall-forming cytoplasm at the point where the bridge passes through.

Figure 49 of the division of the microsporocyte is comparable with figure 22 of the megasporocyte. In both figures one of each pair of attached chromatids has completely disjoined, while the other, with the chromatid from which it failed to separate, forms a taut bridge between the separating masses of chromosomes. A sharply bent fragment is shown near the midpoint of each bridge. In addition to the chromosome fragments, lagging chromosomes also occur (figs. 49–51). Figures 49–62 show chromatid bridges and acentric fragments. In the development of the megagametophyte attenuated chromatid bridges are generally broken and may be absorbed in the cytoplasm. Fragments and lagging chromosomes may be absorbed, or they may persist as micronuclei or as granules.

Accurate determination of chromosome distribution at the first and second meiotic divisions was impossible except in a limited number of very favorably oriented cells which showed no lagging. In figure 21 the chromosomes, including those making up the bridge, are evenly divided, 18–18. In figure 22 the distribution is 19–17, including the chromosomes forming the bridge. Counts were made of 27 megasporocytes at the first meiotic division, and the chromosome numbers of the daughter nuclei were found to vary from 12 to 22, excepting 13 and 20. The mean for the distribution was 17.1. These figures are only approximate, because in the division of the megasporocyte no incoming cell wall marks off the cytoplasm into two compartments, and it is therefore impossible to say with which of the daughter nuclei, if with either, the lagging chromosomes should be counted. If they are credited with neither nucleus, or if only figures showing no lagging are counted, the story of chromosome distribution is still incomplete.

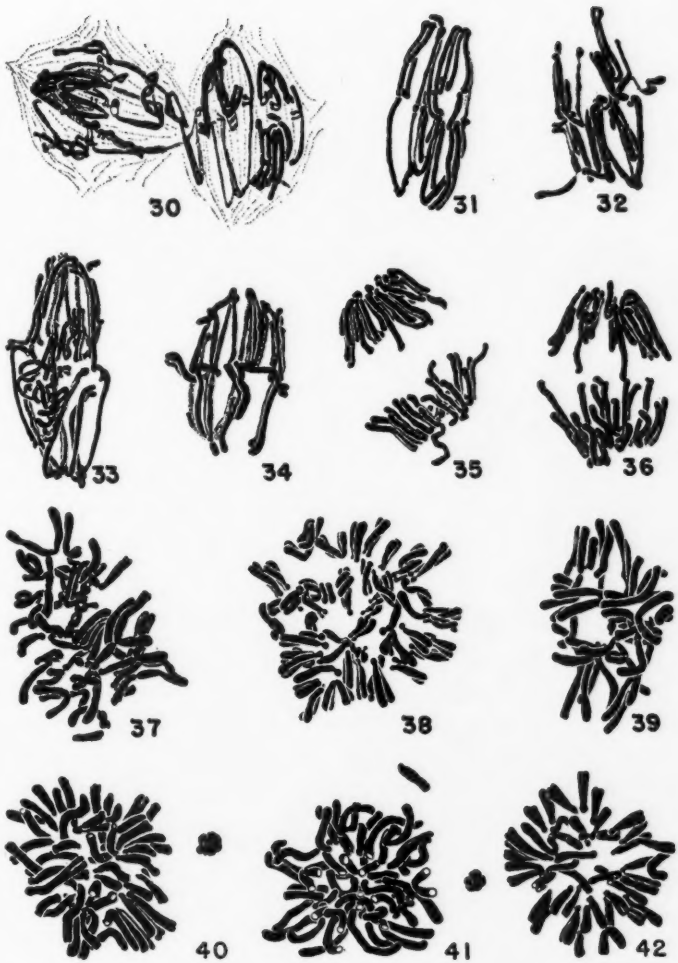
At metaphase of the second meiotic division (figs. 24, 25) few accurate counts could be made because of the manner in which the chromosomes seem to adhere to one another. Further difficulty in making counts was caused by the four chromatids of two adjacent chromosomes with subterminal constrictions being so easily confused with two chromatids of a single chromosome with submedian spindle attachment constriction. With the data from figures that could be counted, however, and from counts at later stages, it seems probable that at the first meiotic division one set of 12 chromosomes goes to one pole and 12 to the other, while the members of the third set are randomly distributed between the two. Of 603 microsporocytes examined, lagging was detected in 47.9 per cent.

Second meiotic division

In the second meiotic division also there is both lagging and bridging. In the lower spindle of figure 26 and in the upper spindle of figure 27 bridging is shown. Approximately midway between the two spindles of figure 27 is shown a lagging chromosome or large fragment in contact with a small accessory spindle. The second meiotic division of the microsporocyte is shown in figure 63. Both bridging and lagging are shown in the lower cell of the two. Typical configurations at metaphase of the second meiotic division are shown in figures 31, 32, and 34. The upper spindle of figure 26 and the lower of figure 27 show the second meiotic division as it more commonly occurs, without bridging and lagging.

In the absence of lagging and bridging the second meiotic division is numerically equational. The effects of lagging, bridging, and fragmentation cause differences in chromosome numbers as great as five in the two nuclei formed as the result of division of a single nucleus. Counts were made of five megasporocytes at the second meiotic division. These limited data are inadequate for drawing conclusions as to the most common assortments, but they serve to illustrate some of the effects of lagging and fragmentation (table 2).

The chromatids, when separated precociously in the first meiotic division, do not divide again in the second. It is possible that owing to unequal distribution of chromosomes the four nuclei formed as a result of the second meiotic division may have different chromosome



FIGS. 30–42.—Fig. 30, division figures of second meiotic division in megasporocyte connected by persisting chromatid bridge; figs. 31–34, late metaphase of second meiotic division; figs. 35, 36, second meiotic division at anaphase; figs. 37–42, chalazal nuclei showing differences in chromosome numbers.

numbers, although this is not usually the case. In the megasporocytes the lagging elements generally roll up in compact masses in the cytoplasm and finally disintegrate or form micronuclei. In the microsporocytes the behavior is much the same except that cell walls may form, splitting off the micronuclei and surrounding cytoplasm to form microcytes. Fragments may persist in the cytoplasm until after the microspores are fully formed.

TABLE 2

DISTRIBUTION OF CHROMOSOMES IN 5 MEGASPOROCYTES AT SECOND MEIOTIC DIVISION

FIRST SPINDLE			SECOND SPINDLE		
1ST NUCLEUS	LAGGING	2D NUCLEUS	1ST NUCLEUS	LAGGING	2D NUCLEUS
19	.....	19	17	1	16
20	.....	20	16	.....	16
22	.....	22	13	.....	13
17	.....	17	12	4	19
18	.....	18	18	.....	18

THIRD AND FOURTH NUCLEAR DIVISIONS

The somatic number of chromosomes is 36 ($3n$), and if each daughter nucleus should receive equal numbers, the nuclei at the first 4-nucleate stage following the second meiotic division would have 18 chromosomes each. Fusion of three of these nuclei at the chalazal end of the megagametophyte would lead to the formation of a nucleus having 54 chromosomes. The constitution of this chalazal nucleus would be, not $3n$ as in the diploid, but $4\frac{1}{2}n$. These theoretical numbers might be found only in those cases in which there is an equal assortment of chromosomes at both first and second meiotic divisions and no loss through lagging and fragmentation. Counts made at the metaphases of the third nuclear division (fig. 28) showed that the chromosome numbers at both chalazal and micropylar ends vary markedly from the theoretical values. The calculated distribution from random assortment was found by expansion of the binomial. As is shown in tables 3 and 4, both chalazal and micropylar nuclei near or at the upper and lower limits of distribution occur

more frequently than would be expected from random assortment, and correspondingly there are less nuclei with chromosome numbers near the calculated mean than would be expected. If there were no loss of chromosomes in the nuclear divisions of the megagametophyte, the expected number of chromosomes in the chalazal nucleus at the metaphase of the third division might be determined by

TABLE 3

CHROMOSOME NUMBERS IN 150 NUCLEI AT MICROPYLAR END OF MEGAGAMETOPHYTE AT METAPHASE OF FOURTH NUCLEAR DIVISION

Chromosome no.	Frequency	Percentage		Percentage $\frac{\text{observed}}{\text{calculated}}$
		Observed	Calculated on random assortment	
3	1	0.66	0.00	.....
12	6	4.00	0.02	166.60
13	13	8.66	0.31	27.93
14	19	12.66	1.61	7.86
15	26	17.33	5.37	3.22
16	28	18.66	12.08	1.54
17	25	16.66	19.33	0.86
18	11	7.33	22.60	0.32
19	7	4.66	19.33	0.24
20	2	1.33	12.08	0.10
21	2	1.33	5.31	0.24
22	5	3.33	1.61	2.06
23	0	0.00	0.31	.....
24	3	2.00	0.02	83.33
31	1	0.66	0.00	.....
38	1	0.66	0.00	.....

Mean chromosome no. of observed nuclei..... 16.82
Mean calculated from random assortment..... 18.00

counting the number in the micropylar nucleus and applying the formula, $72 - M = C$, where M is the number of chromosomes in the micropylar nucleus and C is the number in the chalazal nucleus. At the metaphase of the fourth division the number of chromosomes would be approximately twice that of the third.

A comparison of the observed numbers of chromosomes in the chalazal nuclei with those calculated from random assortment gave the numbers lacking owing to loss through lagging and fragmenta-

tion in the previous divisions. The number lacking, L, may be obtained for the metaphase of the third nuclear division by the formula, $72 - (M + C) = L$. In a study of 28 megagametophytes in which accurate counts could be made at both micropylar and chalazal ends, it was found that 42.85 per cent of the nuclei showed loss of chromosomes, averaging 8.2 chromosomes, or 11.11 per cent loss per cell. This does not mean that this number of chromosomes was actually left out of the nuclei, but that the number lacking owing to loss is as given. Loss of chromosomes in the first division would appear as a deficiency twice as great at metaphase of the third division, and as a deficiency four times as great at metaphase of the fourth division.

Counts were made of chromosomes in 55 micropylar nuclei at metaphase of the third nuclear division and of 150 at the metaphase of the fourth division. In general the distribution was similar in the two stages, the principal difference being a slight shift toward the lower chromosome numbers in the later stage, as shown by a mean of 16.82 chromosomes at metaphase of the third division as compared with 16.25 for the fourth. The difference is probably due to loss of chromosomes during the third division. In all the megagametophytes examined, with the exception of two, the numbers of chromosomes in the micropylar nuclei ranged from 12 to 24 (table 3, fig. 68). In one of these exceptions one nucleus at the micropylar end had 3 chromosomes and the other had 31. In the other case a restitution nucleus possessing 36 chromosomes apparently was formed, as indicated by the presence of two chalazal nuclei and only one micropylar nucleus.

Chromosome counts were made of 47 chalazal nuclei at metaphase of the third nuclear division and of 41 at metaphase of the fourth division. At metaphase of the third division chromosome numbers ranged from 37 to 60, excepting 39 (table 4, fig. 69). At metaphase of the fourth division the range was from 33 to 60, excepting 34 and 35. The mean number per nucleus at metaphase of the third division was 50.04 as compared with 49.85 for the fourth. Except for this slight difference, the distributions of chromosome numbers were similar in the two stages.

If the third and fourth nuclear divisions in the development of the

megagametophyte were mitotic and equational, as has been reported for other species of *Lilium*, the female gametes of the triploid *L. tigrinum* should receive the number of chromosomes occurring in

TABLE 4

CHROMOSOME NUMBERS IN 47 CHALAZAL NUCLEI OF MEGAGAMETOPHYTE AT METAPHASE OF THIRD NUCLEAR DIVISION

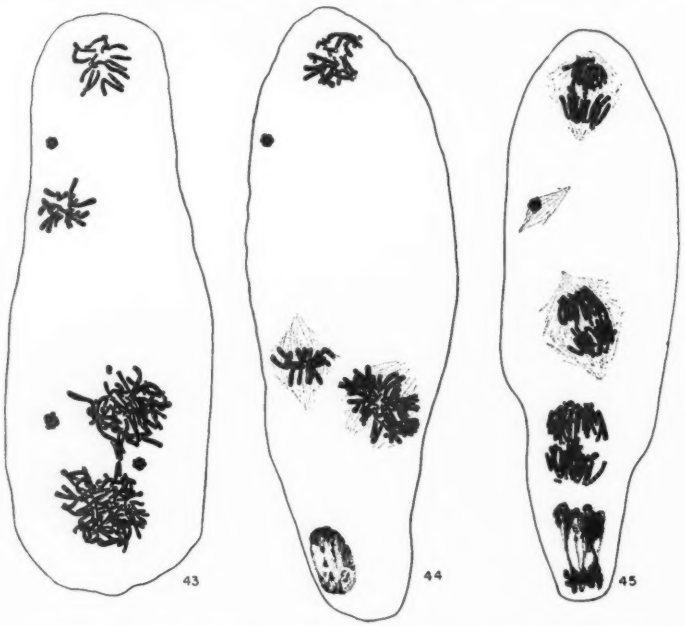
Chromosome no.	Frequency	Percentage		Percentage observed / calculated
		Observed	Calculated on random assortment	
37	1	2.12	0.00	.....
38	2	4.25	0.00	.....
39	0	0.00	0.00	.....
40	1	2.12	0.00	.....
41	1	2.12	0.00	.....
42	2	4.25	0.00	.....
43	1	2.12	0.00	.....
44	2	4.25	0.00	.....
45	1	2.12	0.00	.....
46	1	2.12	0.00	.....
47	3	6.38	0.00	.....
48	1	2.12	0.02	88.33
49	3	6.38	0.31	20.58
50	3	6.38	1.61	3.96
51	2	4.25	5.37	0.79
52	1	2.12	12.08	0.17
53	3	6.38	19.33	0.33
54	6	12.72	22.60	0.56
55	4	8.51	19.33	0.44
56	3	6.38	12.08	0.52
57	3	6.38	5.37	1.18
58	1	2.12	1.61	1.31
59	1	2.12	0.31	6.83
60	1	2.12	0.02	88.33

Mean chromosome no. of observed nuclei..... 50.04
Mean calculated from random assortment..... 54.00

the micropylar nuclei preceding the third nuclear division, and likewise the chalazal nuclei at this stage should have the same number of chromosomes as are later contributed to the polar nuclei which subsequently function in the formation of the primary endosperm nucleus. As shown in figure 29, however, lagging in the third and fourth nuclear divisions of both micropylar and chalazal nuclei may

lower the chromosome numbers of nuclei formed by the divisions following meiosis. The nuclei at the micropylar and chalazal ends of figure 29 actually occurred in different cells, but have been placed together for economy of space.

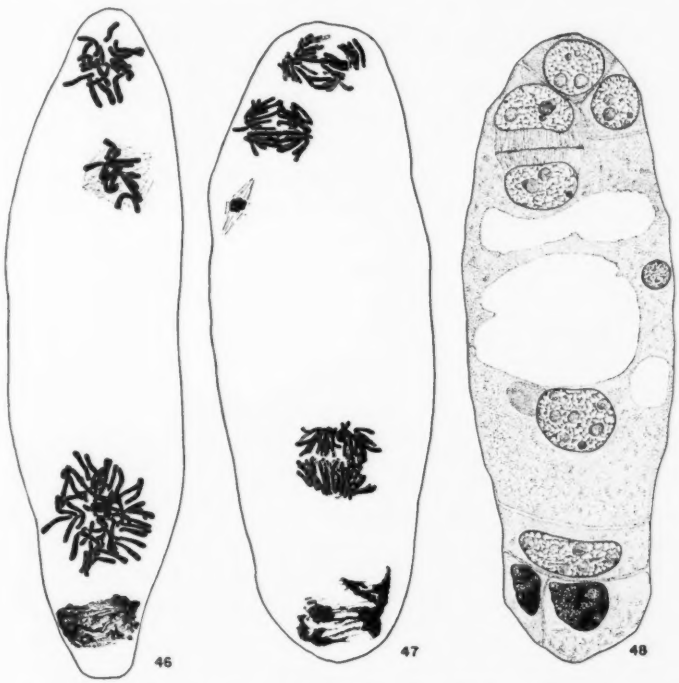
In figures 43 and 44, representing metaphase stages of the fourth nuclear division, compactly knotted lagging chromosomes are



FIGS. 43–45.—Fig. 43, metaphase in fourth nuclear division in development of megagametophyte, showing lagging chromosomes in cytoplasm; fig. 44, metaphase of fourth nuclear division showing widely separated micropylar nuclei; fig. 45, anaphase of fourth nuclear division with micropylar nuclei widely separated.

shown. It is likely that some of these chromatic masses contain more than one chromosome. In figure 45 a chromatic body is shown with a small spindle organized about it. In none of these small spindles have divisions been observed. In figure 48 is a micronucleus which was probably organized from lagging chromosomes lost from the other nuclei. At metaphase of the fourth division the usual

orientation of the four nuclei is as shown in figure 46, with two small nuclei at the micropylar end and two larger ones at the chalazal end. This orientation is generally maintained effectively by the formation of one or more large vacuoles between the two sets of



FIGS. 46–48.—Fig. 46, metaphase of fourth nuclear division as it usually appears; fig. 47, anaphase of fourth nuclear division showing apparently amitotic division of lower chalazal nucleus and a chromatic body in the cytoplasm; fig. 48, 8-nucleate megagametophyte showing egg and two synergids at micropylar end, two polar nuclei and micronucleus toward center, and three antipodal cells at chalazal end.

two nuclei each. Frequently, however, vacuoles may form between the two micropylar nuclei, in which case one is crowded away from the other and forced down into the region of the chalazal nucleus (figs. 44, 45). Still less frequently all four nuclei may be forced by vacuole formation into either the micropylar or the chalazal end.

The lower chalazal nucleus divides apparently amitotically in the fourth division (figs. 44–47), while the upper chalazal nucleus forms a well-spaced metaphase plate (fig. 46). It was at this stage that counts were made. The 8-nucleate megagametophyte is represented in figure 46, in which a small micronucleus is also shown.

Discussion

It has been reported that the diploid *Lilium tigrinum* sets seed readily when self-pollinated, but that the triploid form rarely does (**32**). STOUT (**38, 39**), who collected over 200 types of *L. tigrinum* which produced no seeds following self-, close-, and inter-pollination, states that the single-flowered clones of this species known previous to 1932 have been completely fruitless to cross- and self-pollination. SATO (**33**) reported chromosome counts of the progeny of self- and inter-pollinated plants of *L. tigrinum* and found a wide variation in chromosome numbers, ranging from 24 to 39, excepting 31. He concluded that these seedlings must have resulted from the fusion of gametes with irregular numbers of chromosomes produced by an autotriploid mother plant. From a consideration of the chromosome numbers of the progeny, he presumed the existence of gametes ranging from 12 to 27, with 12 and 14 chromosomes in nuclei occurring most frequently. He also reported the occurrence of fragments, and postulated a structural change in some of the chromosomes.

The plants used in the present investigation have been observed for a number of years and have never been seen to produce capsules following self- or inter-pollination. That sterility is due principally to self-incompatibility in the clones of the triploid *L. tigrinum* rather than to failure of formation of functional gametes is shown by the production of both capsules and viable seeds when cross-pollinated with other species, using the triploid *L. tigrinum* as seed parent in some instances and as pollen parent in others. Table 5 lists successful crosses taken from several different reports.

No cytological work has been reported on any of the *L. tigrinum* hybrids, but in view of the present investigation it seems probable that such hybrids are likely to be subject to irregularities in meiotic divisions and to show considerable variations in somatic numbers. Data given by CHANDLER, PORTERFIELD, and STOUT (**11**) on the

division of the microsporocyte, and additional data obtained in the present investigation on the divisions of both the microsporocytes and the megasporocytes of *L. tigrinum*, indicate that a set of 12 chromosomes passes to each pole in the first meiotic division, and that members of the third set are more or less randomly distributed

TABLE 5

LILIUM TIGRINUM CROSSES

LILIUM SEED PARENT AND POLLEN PARENT	INVESTIGATOR AND DATE
tigrinum×maximowiczii	Preston (**25**), 1925 Stout (**38**), 1926
tigrinum var. splendens×maximowiczii	Beal (**4**), 1937
tigrinum×sutchusense	Stout, 1926
tigrinum×warleyense	Preston (**26**), 1933
tigrinum×willimottiae	Preston, 1933
tigrinum var. splendens×willimottiae	Beal, 1936
tigrinum×davidii	Preston, 1933
tigrinum×amabile	Stooke (**37**), 1937
tigrinum×elegans	Stooke, 1937
tigrinum×elegans wallacei	Stout, 1926
tigrinum×pseudotigrinum	Preston, 1933
tigrinum×tigrimax	Preston, 1933
tigrinum var. splendens×tigrimax	Beal, 1937
tigrinum var. splendens×auratum	Beal, 1937
tigrinum var. splendens×batemanniae	Beal, 1936
tigrinum var. splendens×regale	Beal, 1936
tigrinum var. splendens×superbum	Beal, 1936
tigrinum var. splendens×leichtlinii	Beal, 1937
speciosum×tigrinum	Preston, 1933
bulbiferum croceum×tigrinum var. splendens	Stooke, 1937
henryii×tigrinum	Preston, 1933
henryii×tigrinum var. splendens	Beal, 1936
speciosum×tigrinum	Preston, 1933
davidii×tigrinum ($2n$–24) var. fortunae	Preston, 1933
maximowiczii×tigrinum	Preston, 1933
regale×tigrinum	Preston, 1933
regale×tigrinum var. splendens	Beal, 1937
superbum×tigrinum var. splendens	Beal, 1936

between the two poles. Elimination of chromosomes causes a general shift toward the lower numbers from the distribution expected on random assortment. The result is that nuclei with n number of chromosomes or with $n + 1$ or $n + 2$ occur with much greater frequency than would be expected from random assortment. That loss of chromosomes through lagging is not entirely responsible for the greater than expected frequencies of nuclei with low chromosome

numbers is shown by the occurrence of many more nuclei than is expected at the upper limit of distribution. Even after having the chromosome numbers lowered by lagging and fragmentation, nuclei at or near the diploid end of the distribution occur in greater than expected frequencies (figs. 68, 69; tables 3, 4).

After allowance is made for the general shift to the left, the distribution approaches that expected from random assortment, except that fewer nuclei are found at or near the mean and more at or near the haploid and diploid limits than expected. A departure from calculated distribution similar to this has been reported for several triploids. BELLING (**5**) for *Canna*, DERMEN (**16**) for *Petunia*, BELLING and BLAKESLEE (**9**) and SATINA and BLAKESLEE (**31**) for *Datura* have reported that triploid plants of these genera show chromosome distribution at meiosis similar to that here found for *L. tigrinum*.

In later stages of development in the male gametes in triploids a wide variation in chromosome numbers has been reported. SATINA and BLAKESLEE on *Datura*, DARLINGTON (**15**) on *Hyacinthus*, LEVAN (**20**) on *Allium*, CAPINPIN (**10**) on *Oenothera*, and NAGAO (**23**) on *Narcissus* reported nuclei with all chromosomes, ranging from n to $2n$. In most of the investigations nuclei with lower chromosome numbers were found to be more numerous than those at the other limit of distribution. Not in all instances were the numbers of nuclei at or near the limits of distribution greater than expected.

The distribution of chromosomes at the metaphases of both the third and fourth nuclear divisions is similar in micropylar and chalazal nuclei, except that the effects of lagging and fragmentation are trebled in the chalazal nuclei, and as a result the shift to the lower chromosome numbers is more marked than in the micropylar nuclei (figs. 68, 69). Lagging in the nuclear divisions following meiosis has not been reported in previous investigations. In *L. tigrinum* such lagging occurs rather infrequently as compared with that in the meiotic divisions, and is possibly related to the chromosome unbalance and the existence of dicentric chromosomes and fragments. Actual counts were not made of chromosomes in the female gametes, but numbers at the metaphase of the fourth nuclear division should represent those counts rather accurately, since numbers would be modified so slightly in the fourth division.

Differential assortment of chromosomes of the type favoring the production of haploid and diploid gametes, as it occurs in *L. tigrinum*, may be explained if it is found that certain homologous chromosomes tend to pass together to the same pole. Behavior of this type has been reported in *Drosophila* by STURTEVANT (40) and in *Rosa* by HURST (19), but neither of these cases is comparable with that of *L. tigrinum*. In *Rosa*, chromosomes which failed to pair went together to the same pole. As shown in table 1, there is almost complete trivalency in the triploid *L. tigrinum*, indicating that the three sets of chromosomes making up the somatic number are very similar. There is, nevertheless, evidence that one set of 12 chromosomes may differ from the other two in regard to the structure of some of its members. Failure of correspondence in all chromomeres of a set of three homologous chromosomes has been mentioned. In previous investigations SATO (33) and CHANDLER, PORTERFIELD, and STOUT (11) also reported such apparent lack of strict homology in the prophases of the microsporocyte. They gave no definite suggestion as to the cause of the irregularity.

In the present investigation at early diakinesis of the megasporocyte three chromosomes were observed associated as shown in figure 66*u*, and as interpreted in figure 66*v*. Such configuration may result from a cross-over in an inverted segment distal to the centromere in the first of the three chromosomes. The break in the chromosome is supposed to have occurred at the point *x*, and an apparent lack of symmetry at this point—which corresponds in position to the end of the second chromosome—gives additional evidence in support of this supposition. The occurrence of chromatid bridges and fragments at the anaphase and telophase of both the first and second meiotic divisions adds confirmatory evidence for the existence of an inverted segment in at least one of the chromosomes.

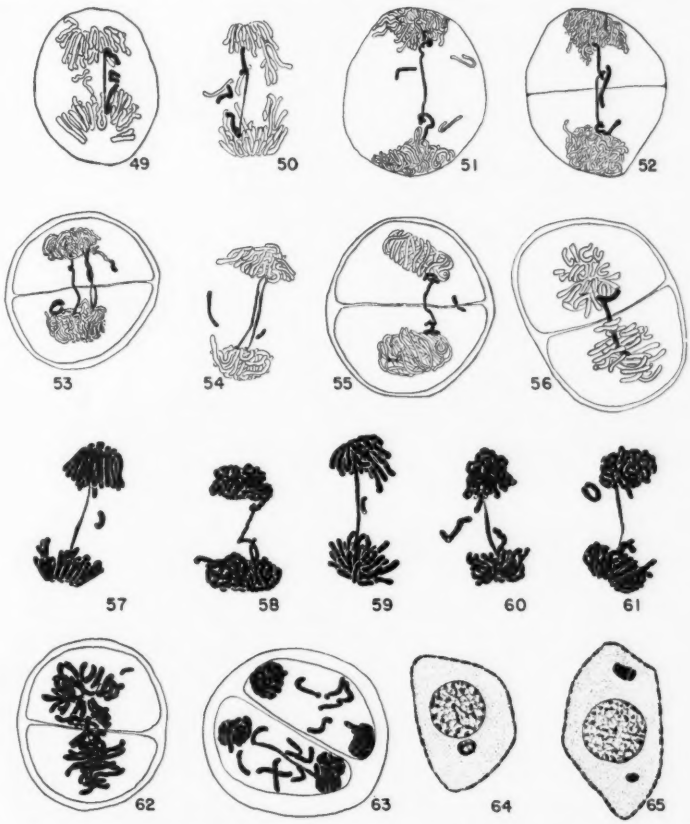
The relationship of bridging and fragmentation to crossing-over in an inverted segment distal to the centromere of one of the chromosomes is shown in figure 66, columns I, II, III, and IV. In the first three columns only two of the three homologous chromosomes are considered. In column I is diagrammed the possible sequence of events after the formation of one chiasma at pachytene (*c*), followed by bridge formation and fragmentation in metaphase and anaphase (*d*, *c*). In column II a similar sequence is shown following formation

of two chiasmata. The final configuration is the same in both cases. The acentric fragment may vary in length, depending on the distance from the end of the chromosome at which the chiasma in the inverted portion forms. Nuclei showing bridges and acentric fragments which may have been formed according to the plan of columns I and II are shown in figures 21, 22, 23, 30, and 49–60.

In column III of figure 66 is shown a complementary cross-over, resulting in the formation of two bridges and two acentric fragments. Cases of double bridge formation are shown in figures 53 and 54, and in *D* and *E* of figure 67. In column IV of figure 66 and in figure 67 are presented types of separation possible when three homologous chromosomes are associated as shown in figure 66*u*. It is probable that the two chromosomes joined by a chromatid connection tend to move together to the same pole in the first meiotic division, in which case the bridge is not evident until the second meiotic division. It may then occur in one of the two dividing nuclei (figs. 26, 27, 63).

If the two centromeres of the bridge-forming chromosome should always pass to opposite poles in the second meiotic division (fig. 67*J*, *K*), the ratio of bridges in the first and second meiotic divisions would form an index measuring the degree of differential or random assortment at the first division. But if the two centromeres should pass to the same pole, as in figure 67*L*, which is probably more likely, then the number of bridges appearing stretched from pole to pole in the second meiotic division would represent only a portion of the dicentric chromosomes actually present. In order to compute the degree of differential or random assortment in the first meiotic division, the percentage of bridges apparent in the second meiotic division should be multiplied by the ratio of dicentric chromosomes present to those apparent as bridges. For example, following random assortment of the two centromeres of the dicentric chromosome at the second meiotic division, the ratio of dicentric chromosomes present to those apparent as bridges would be 2 to 1. If, as is likely, the assortment is differential in favor of the two centromeres passing to the same pole, the ratio would be more than 2 to 1. Counts of chromosomes in the microsporocytes show an average of 17 per cent bridging in the second division as compared with 11.6 for the

first. If the percentage of bridges apparent in the second division is multiplied by two to allow for random assortment, the ratio of



FIGS. 49–65.—Figs. 49, 50, first meiotic division of microsporocytes, showing lagging and bridging; figs. 51, 52, 55, bridging and acentric fragments in microsporocytes; figs. 53, 54, double bridging; figs. 56, 62, bridges persisting at metaphase of second meiotic division; figs. 57–61, late anaphase and telophase of first meiotic division, showing bridging and acentric fragments; fig. 63, lagging chromosomes and bridging in second meiotic division; figs. 64, 65, microspores showing chromatic masses in cytoplasm.

bridges in the first meiotic division is 11.6 to 2×17, or 11.6 to 34. This means that two chromosomes attached by a dicentric chroma-

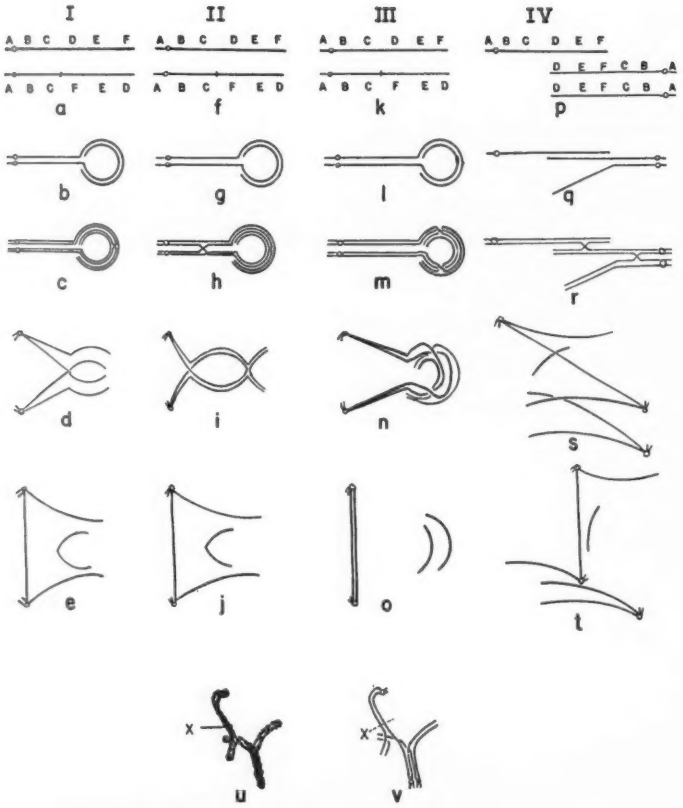


FIG. 66.—Diagrammatic representation of bridging as result of cross-over in inverted segment. Column I: *a*, two chromosomes showing reversal in segment of lower of the two; *b*, zygotene; *c*, pachytene with one chiasma in inverted portion; *d*, *e*, chromatid bridge and acentric fragment. Column II, sequence following formation of two chiasmata. Column III, double bridging following complementary cross-over. Column IV, sequence of events expected accompanying separation of trivalent chromosomes, one of which possesses inverted segment; *u*, observed trivalent association diagrammed in *v*, on which column IV is based.

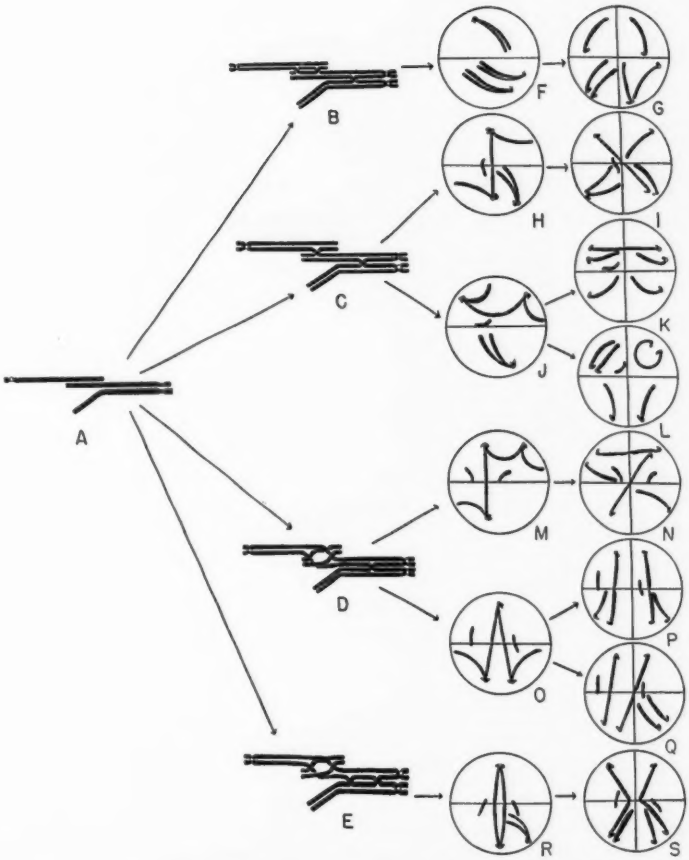


FIG. 67.—Possible meiotic distribution of three homologous chromosomes, one of which shows inverted segment: *A*, three homologous chromosomes arranged as in fig. 66, column IV; *B*, *F*, *G*, reciprocal crossing-over in which no bridges are formed; *C*, *H*, *I*, bridging at first meiotic division persisting until after the second; *C*, *J*, *K*, bridging at second meiotic division; *C*, *J*, *L*, no bridge formation following passage of the two centromeres of dicentric chromosome to same pole at both first and second meiotic divisions; *D*, *M*, *N*, *O*, *P*, *Q*, some possible arrangements of chromosomes following double bridge formation joining three chromosomes; *E*, *R*, *S*, double bridging resulting from complementary cross-over involving only two homologous chromosomes.

tid at the first meiotic division pass together to the same pole approximately three times more often than to opposite poles. If there is a similar differential assortment of centromeres of the dicentric chromosomes at the second meiotic division, only those whose centromeres pass to opposite poles are apparent as bridges, and the

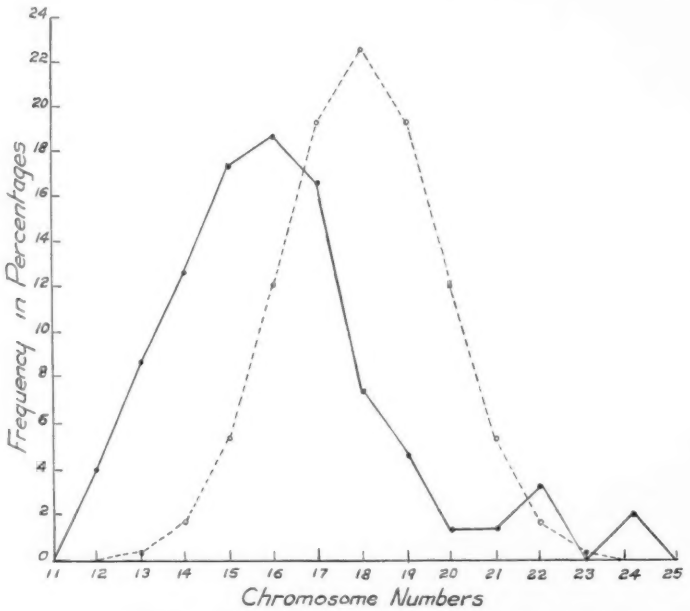


FIG. 68.—Graph showing distribution of chromosome numbers in 150 nuclei at micropylar end of megagametophyte, at metaphase of fourth nuclear division. Broken lines indicate distribution calculated from random assortment; solid lines represent observed frequencies.

index of differential assortment at the first meiotic division is considerably more than 3 to 1.

It is also possible that infrequently three homologous chromosomes become connected by two chromatid bridges (fig. 67*D*, *M*, *O*). Possible results of random assortment of the centromeres of the bridge-forming chromatids are shown in figure 67*N*, *P*, *Q*. More often the centromeres may all pass to the same pole, since all are

connected by bridges, and this would result in a decided differential assortment favoring formation of haploid and diploid nuclei and decreasing the number near the mean expected from random assortment. The nuclei with smaller chromosome numbers formed in this way would be non-functional as gametes, since they would not have a set of 12 different chromosomes. Half of the nuclei formed following the formation of two bridges (fig. 67*R*, *Q*, *S*) would also have an incomplete set of entire chromosomes. Although the oc-

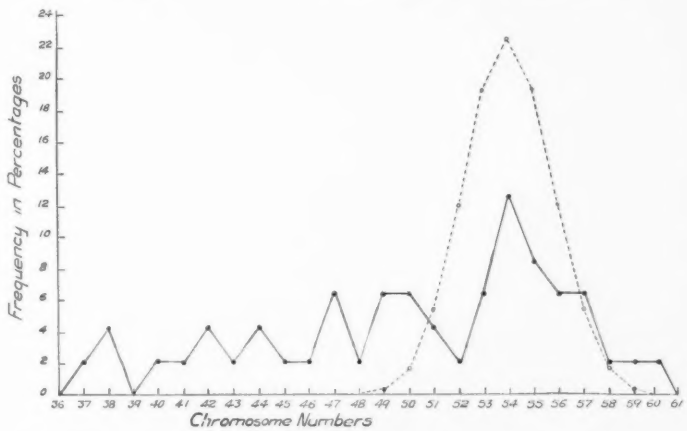


FIG. 69.—Graph showing distribution of chromosome numbers in 47 chalazal nuclei of megagametophyte, at metaphase of third nuclear division. Broken lines indicate distribution calculated from random assortment, solid lines represent observed frequencies.

currence of three homologous chromosomes joined by chromatid bridges may result in a more marked differential assortment in the particular cell in which it occurs than does the occurrence of two chromosomes so joined, the latter condition is so much more frequent that it probably influences differential assortment as a whole more. The numbers of haploid and diploid nuclei resulting from differential assortment would be approximately equal were it not for lagging and fragmentation, which decrease the number of nuclei with high chromosome counts and increase the number with low counts. It is therefore probable that the number of diploid progeny

of a cross between *L. tigrinum* and a diploid form would exceed that expected. Selective growth favoring haploid gametes at fertilization would also tend to produce diploid progeny. Evidence that the proportion of diploid plants resulting from triploid × diploid crosses exceeds that expected from random assortment has been presented by BELLING and BLAKESLEE (**9**) in their work with *Datura* and by VAN OVEREEM (**43**) with *Oenothera*.

The presence of an inverted segment in at least one of its 36 chromosomes places *L. tigrinum* var. *splendens* in the structural hybrid class, notwithstanding the high degree of trivalency at diakinesis. Evidence for structural changes in chromosomes has previously been reported for several species of *Lilium*. RICHARDSON (**27**) reported inversions and duplications in the hybrid *L. martagon* × *L. hansonii*. Failure of pairing has been reported for other species by HALL and MATHER (**18**), BELLING (**8**), SANSOME and LACOUR (**28**), and SATO (**33**). It is possible that many of the recognized species of *Lilium*, including *L. tigrinum*, are of hybrid origin. The prevalence of structural hybridity, although not conclusive evidence for interspecific hybridity, is at least of suggestive significance.

Bridging and fragmentation have been reported in structural hybrids of other genera. Recently WOODS (**44**), in an investigation of the triploid tulip Inglescomb Yellow, reported both bridging and fragmentation. As is true of *L. trigrinum*, the Inglescomb Yellow tulip showed a high degree of trivalency at diakinesis. In another recent investigation EMSWELLER and JONES (**17**) reported bridging in a hybrid between *Allium cepa* and *A. fistulosa*.

A close parallelism is evident between meiotic irregularities associated with structural hybridity due to inversion and irregularities commonly reported in triploids at meiosis. Among these irregularities are formation of univalents, which results in lagging chromosomes and subsequent production of micronuclei and microcytes. A second case of parallelism occurs in the type of departure from random assortment of chromosomes. The formation of bridges has also been reported in both types. These similarities in behavior suggest that structural hybridity may occur in triploids more often than has been supposed.

Summary

1. Development of the megagametophyte in *Lilium tigrinum* var. *splendens* follows the general plan reported for the other members of the genus and for *Fritillaria* and *Tulipa*.

2. At metaphase of the first meiotic division 36 chromosomes generally occur in 12 trivalent groups, although bivalents and univalents may occur.

3. Distribution of chromosomes at the first meiotic division approaches random assortment, except that there are marked increases toward the $1n$ and $2n$ limits of distribution.

4. The chromatids of lagging chromosomes may separate precociously following the first meiotic division, after which they may pass tardily to the poles or remain in the cytoplasm between the two nuclei.

5. Lagging chromosomes and fragments eliminated from nuclei may disintegrate or persist as micronuclei.

6. Elimination of chromosomes through lagging and fragmentation in the meiotic divisions and to a less marked degree in the divisions following meiosis produces a general shift in the distribution toward the lower chromosome numbers.

7. Lagging chromosomes and bridging occur in the divisions following meiosis in the development of the megagametophyte.

8. The occurrence of chromatid bridges and fragmentation of chromosomes at meiosis results from crossing-over in an inverted segment of one of the chromosomes.

9. Crossing-over in an inversion results in the formation of dicentric chromosomes at meiosis in a triploid and thus favors the formation of haploid and diploid gametes.

10. There is close parallelism between meiotic irregularities found in structural hybrids and irregularities common to triploids.

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STRUCTURE OF FOSSIL STEM OF PITYEAN AFFINITY FROM THE REED SPRINGS FORMATION OF MISSOURI[1]

J. E. CRIBBS

(WITH NINETEEN FIGURES)

Introduction

The petrified stem described in this paper is from the Reed Springs Formation of the Mississippian Series. Three other stems have been described (**4, 5, 6**) from the same horizon. Two of those previously reported were found imbedded in chert, which forms more or less continuous seams in the limestone of this formation. This stem was found lying free among an accumulation of fragments which had broken up under the influence of weathering after the limestone had dissolved.

The specimen probably represents part of the main trunk of a small tree which was broken transversely by fractures subsequent to fossilization. The portion found (fig. 14) was about 35 cm. long, 9.5 cm. wide, and 6.5 cm. thick. Erosion has so affected the stem as to remove all structures external to the secondary wood, and an unknown amount of the latter has weathered from the surface, leaving a zone of unequal thickness. The stem is straight, approximately uniform in thickness, and without visible branches.

The petrifaction is siliceous, like that of other stems from chert seams of the Reed Springs Formation. There is a further resemblance in the bruised condition of the secondary wood, a feature which is particularly evident in sections. Apparently a certain amount of decay took place before infiltration, so that pits of both primary and secondary elements are poorly preserved, and are clearly visible in limited areas only.

Compression flattened the stem slightly, crushed part of the pith cells, produced four longitudinal fractures, and affected all of the

[1] This investigation was aided by a grant from the American Association for the Advancement of Science, awarded through the Missouri Academy of Science.

tissues in local areas. Spherulites occur throughout the specimen. About the margin of the pith, and especially in the primary bundles, there is an accumulation of dark materials which at places obscures the cell arrangement.

SECTIONS PREPARED.—In all, fifty-five sections were prepared, all cut on the petrotome. The peel method was not used because of partial decay of the organic material before petrifaction. After cutting the stem transversely, one of the two pieces was broken along a fracture which extended lengthwise near the center of the pith. The cut end surface of one piece was polished to show the general topography of the transverse sectional area. A large thin section was prepared (a composite) to show the number and arrangement of primary bundles. This included all the pith and the first growth zone of secondary wood surrounding it.

Twelve transverse sections were cut in series from a block 28 mm. long and 23 mm. wide, which included pith. These were prepared to determine the arrangement of primary bundles at the nodes, and to observe the general features of leaf trace emission. Because of certain irregularities that appeared in this series, it was supplemented by thirty-three tangential sections cut so as to show all the outgoing traces in a segment of stem 18 cm. long which included 5 cm., or about two-fifths of the pith circumference. Eighteen of these are thin sections cut between the pith margin and a point in the secondary wood about 4 mm. beyond the terminus of leaf trace bundles. Fifteen are polished surfaces of blocks cut about 2 mm. from the pith, and were prepared to determine the relative position of outgoing traces, whether the traces are single or double, and if possible to secure information regarding phyllotaxis. The remaining sections include tangential cuts of the pith and primary bundles; transverse sections of secondary wood; and radial sections of reparatory strands, outgoing traces, and secondary wood.

Observations

PITH

Maximum dimensions of the pith as seen in transverse section are 50 mm. by 22 mm. (figs. 7, 15). In the central region is a band of crushed cells lying in the plane of greatest diameter, which apparent-

ly was parallel to the bedding plane of the stem before it was dissociated from the chert matrix. This band is about 5 mm. wide. Fine compression lines form a fan opposite each end of the crushed zone and extend outward to the secondary wood. Within this region the pith cells are somewhat distorted and structural features are partially obscure. Surrounding the central region of compressed cells, the pith varies from 5 mm. to 1 cm. in width and is composed of cells which are elongated radially, except for about 1 mm. adjoining the secondary wood where their greatest dimension is in the vertical axis and the radial elongation is less evident.

There are no sclerotic tissues or secretory ducts of any kind in the pith, which is perfectly continuous except for an irregular longitudinal fissure that developed where the fracture planes intersect near the center of the stem.

The boundary between pith and secondary wood, as seen in transverse sections, is not a smooth curve. It is characterized by shallow embayments five or six of which are visible in transverse section (fig. 7). These may be due in part to alteration during compression, but for the most part they represent embayments attending leaf trace emission. They affect the inner growth zone of secondary wood, beyond which the successive zones appear as smooth concentric lines since they are not traversed by outgoing leaf trace bundles.

The average dimensions of pith cells are as follows: tangential width 118.6 μ, radial width 264.6 μ, and length (height) 142.8 μ. Within 1 mm. of the margin the average dimensions are 84 μ tangential width, 142 μ radial width, and 168 μ height.

Primary bundles

In a transverse section of the stem ninety-one primary bundles are visible in the pith a short distance from the margin, and seven others are in contact with the secondary wood (fig. 7). The latter have not at that level given off a reparatory strand. Probably two or three additional bundles were present and were lost in the development of the fractures.

The bundles are submarginal in position and vary considerably in their distance from the pith margin. Most of them are 1 mm. or more from the wood and some are 2–3 mm. distant. They are dis-

tinctly isolated. There is not the tendency to become confluent as in the semi-herbaceous Calamopityeae (**10,13**). The average distance of separation is about 1 mm., which is approximately five times their tangential diameter.

Where unaffected by compression, the primary strands are more or less circular in outline as seen in transverse section. Some of them are elliptical, with the greater dimension in the radial plane (fig. 12).

The bundles are typically mesarch with the protoxylem approximately central. There is some variation, however, so that occasionally they approach the endarch arrangement. The endarch tendency occurs only in the region where they curve out from the pith to the secondary wood, and where they divide tangentially to form leaf trace and reparatory strand (fig. 5).

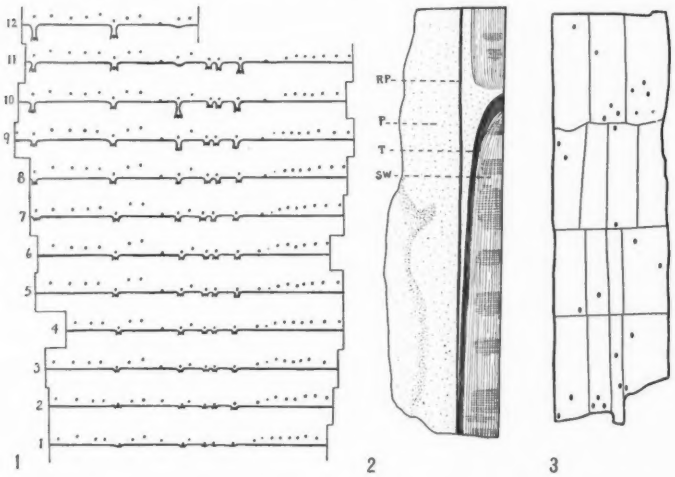
Those bundles which lie 1 mm. or more from the wood are within the zone where pith cells are radially elongated, and cells adjoining the strand commonly radiate from it as a focal point, or they may not be so elongated on the tangential borders. Nearer to the pith margin, cells adjacent to the bundles are not conspicuously elongated but tend to conform to the neighboring arrangement, being slightly longer in the vertical axis and approximately round in transverse section.

In the series of twelve transverse sections cut from a block 28 mm. long and 23 mm. wide (fig. 1), a maximum of twenty-three primary bundles appear in section 9. In this portion of the stem the bundles all follow a vertical course and there are no indications of lateral connections. A submarginal bundle turns out to the pith margin a considerable distance below the node (probably about 3 cm.), as seen in figure 4, and coincident with the formation of an embayment in the wood it divides tangentially to form an outer leaf trace and an inner reparatory strand (figs. 2, 6). The reparatory strand continues vertically, lying directly opposite the trace, and gradually comes to lie deeper in the pith. Each emerging trace shows the same structural arrangement.

In the composite section of the entire pith cylinder (fig. 7) there are five double bundles which lie at considerable distance from the secondary wood. Therefore branching of the submarginal bundles is known to have occurred sparingly at other places than the nodes.

The primary bundles are slightly larger in the upper part of their course, averaging 190 μ (fig. 5), but the enlargement does not attain the dimensions described for the various Calamopityeae. As shown in figures 8 and 11, in the outgoing trace, where largest (400–600 μ), they resemble those of *Callixylon* (1).

The size, large number, and submarginal position of the bundles agree with the condition originally described for *Pitys antiqua*. There

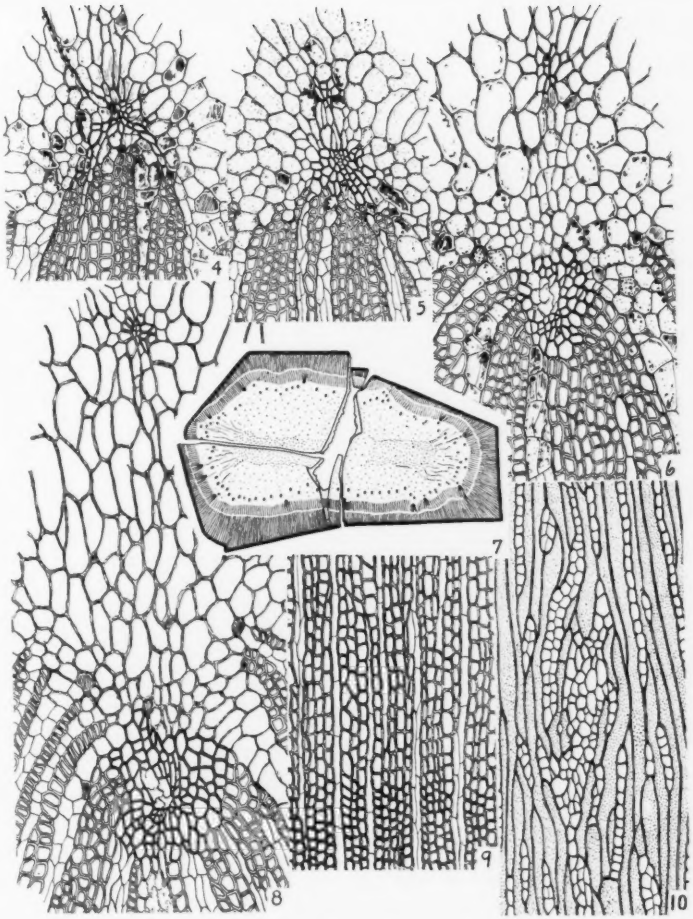


FIGS. 1–3.—Fig. 1, reconstruction of twelve transverse sections from a block 28×23 mm., showing arrangement of primary bundles in relation to outgoing traces. Note vertical course and lack of interconnections. Fig. 2, reconstruction of radial section through reparatory strand, trace, pith, and secondary wood (*rp*, reparatory strand; *t*, trace; *p*, pith; *sw*, secondary wood). Fig. 3, reconstruction of fifteen blocks, representing fragment of stem 5×18 cm. at pith border; traces shown as black elliptical dots; note paired arrangement except for two clusters.

are, however, no deeply imbedded medullary bundles such as have been described in *Archaeopitys eastmanii* (11, 12) and more recently in all the known species of *Pitys* in which primary tissues are preserved.

LEAF TRACES

A marginal bundle divides tangentially to form an outer leaf trace and an inner reparatory strand (fig. 5). The trace bundle, which is the larger, begins at once to sink into the secondary wood. It forms



FIGS. 4–10.—Fig. 4, transection of primary bundle (mesarch) where it turns out from pith to inner border of secondary wood, below point of division into reparatory strand and trace; ×53. Fig. 5, formation of reparatory strand and trace; latter is the larger and lies in contact with secondary wood; ×53. Fig. 6, beginning of trace emergence; trace somewhat enlarged with a few centrally placed parenchyma cells; excentrically mesarch trace; ×53. Fig. 7, composite showing transverse section of entire pith, first growth zone of secondary wood, and primary bundle arrangement; ×$\frac{1}{2}$. Fig. 8, later stage of trace emission showing embayment in secondary wood, mesarch trace, and reparatory strand deep in pith; ×53. Fig. 9, transection of secondary wood showing border between summer and spring wood; ×50. Fig. 10, tangential section cut about 2 mm. from terminus of leaf trace, showing slight distortion of secondary elements; ×53.

an acute angle with the stem axis, and does not reach the outer edge of the first growth zone of secondary wood (3 mm. thick) until it has passed through a vertical distance of 2–2.5 cm. As a trace approaches the outer margin of the first growth zone, it curves sharply and assumes a position almost but not quite horizontal.

A small group of parenchyma cells is included with the primary xylem of the trace (figs. 6, 7). The mesarch arrangement typical of the primary bundles is continued in the trace, where the protoxylem lies immediately outside the parenchyma, and a second protoxylem center appears to be present in some instances, when it lies on the centripetal side of the parenchyma cells. There is a strong tendency for the centrifugal metaxylem to develop in radial alignment with the secondary elements, a feature characteristic of certain Cordaiteae (9).

As the trace emerges, a fan of secondary wood is carried out on the abaxial side, but so far as can be determined does not develop adaxially. At the outer border of the wood, an outgoing trace with its secondary elements has an average diameter of about 600 μ. Some of them do not exceed 400 μ. There is very little distortion of tracheids or rays attending the emergence of traces. On the abaxial side of the trace, where such distortion is commonly greatest, the rays are slightly wider and somewhat shorter than elsewhere; opposite their terminus in the wood secondary elements close over the break quickly, so that the tracheids appear quite normal about 2 mm. external to that point (fig. 10). Since the traces terminate at the outer border of the first growth zone, it is probable that the leaves were shed at the close of a growth season and were replaced by others at a higher level when growth was resumed.

There is a peculiar grouping of certain traces as seen in the series of transverse sections (fig. 1), where they lack the regularity normal to a spiral arrangement. Six traces pass out in the block, all emerging at about the same level. Their spacing in relation to the primary bundles is also irregular. Between the two traces shown at the left of the figure there are four intervening bundles; between the second and third there are three bundles; between the third and fourth there is one bundle; while the fourth, fifth, and sixth are derived from successive strands. Two bundles, one on each side of the

clustered traces, are in contact with the wood and presumably emerged slightly higher in the stem. None of the seven shown at the right have approached the wood preliminary to emerging. One bundle, the seventh from the right of section 2, diminishes in size upward and apparently terminates blindly, as it consists of a single tracheid in the last section and holds to a vertical course. Since its origin is not shown in this series, there is some doubt, but it probably is derived from a neighboring strand by division such as is seen to occur in the composite section previously referred to. If this is the true interpretation, it may represent in a much reduced form the condition described for *Archaeopitys eastmanii* (**11, 12**).

Because of the peculiar grouping of traces in this portion of stem, additional information was sought by cutting into suitable blocks a considerable portion of the stem obtained by breaking it along the main longitudinal fracture. These blocks, fifteen in number, were then so cut on the tangential plane as to show the traces on their way through the first growth zone of secondary wood. Figure 3 shows the relative position of the blocks and represents about two-fifths of the pith circumference. The traces, as shown in the figure, mark the relative levels at which they passed from the wood into the cortex, but their horizontal spacing corresponds to their distance apart at the pith margin where they connect with reparatory strands.

With the exception of two groups, including nine traces above and five below, they appear to be paired in arrangement, one lying at a higher level than the other. This arrangement resembles that described for *Pycnoxylon* (**5**), except that in this stem the traces are separated by one to four intervening bundles. It is possible that on their way through the cortex the traces changed their relative position, so that each entered a separate petiole. This appears doubtful, and the grouping can best be explained as a paired arrangement.

In regard to the two groups of traces there is considerable obscurity. They may represent bundles that passed out into a cauline appendage which may have been a fruiting stalk such as occurs in the Cordaiteae (**9**). The close grouping cannot be explained as due to excessive local compression that affected their relative positions, for the five shown at the upper right in the figure were in a region

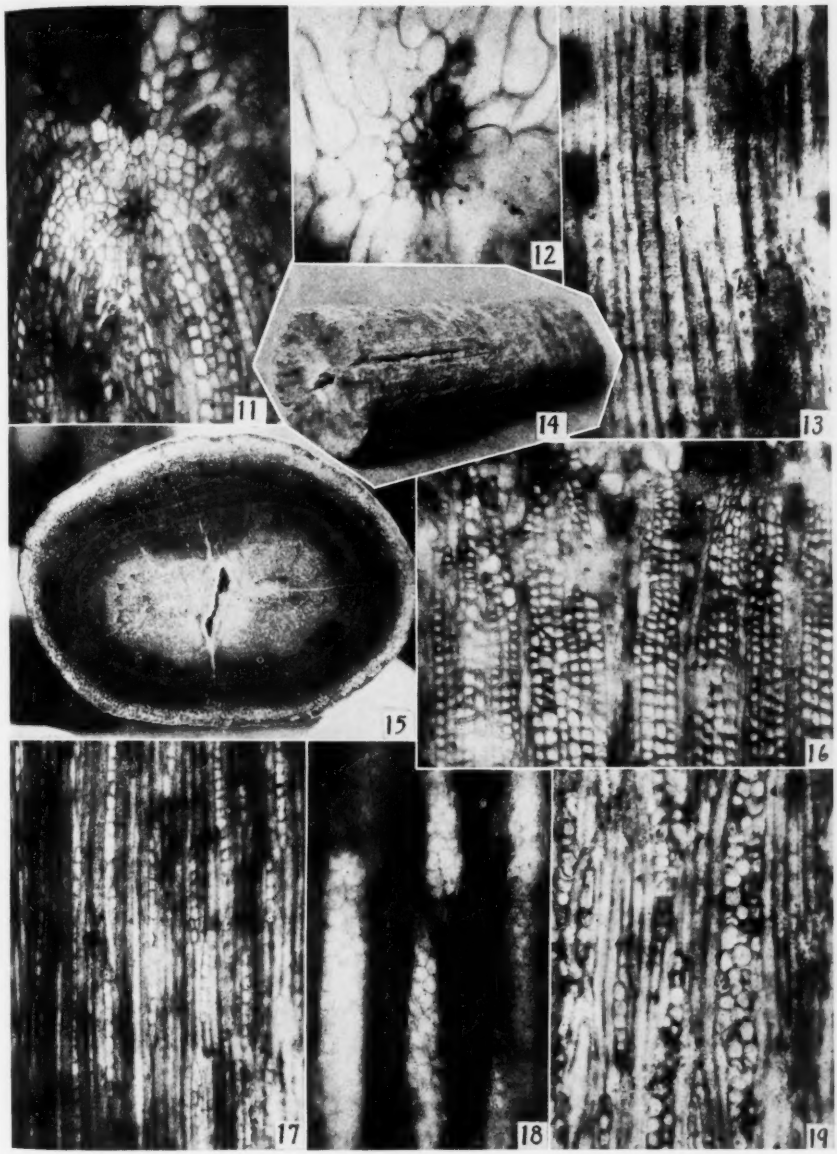
not affected by compression, and the group seen in the transverse series are shown to be derived in part from successive primary bundles. If they passed out into a fruiting stalk, that structure must have been herbaceous and caducous, for the bundles terminate in the secondary wood at the outer border of the first growth zone in the same manner as the others and there is no evidence of the secondary wood being involved, except for a small amount which is incorporated in the trace bundle. They are closed over by the second growth zone exactly like the traces. It is not probable that these grouped traces connected with buds, for in that instance they would doubtless bear a more consistent relation to the leaf traces, and if they extended into lateral vegetative branches there would be an extensive involvement of secondary wood. That these strands all supplied leaves is scarcely tenable, for obviously it would imply local irregularity in spiral arrangement. To the writer the most plausible explanation is that regarding a fruiting appendage.

The leaf arrangement is obscure, but apparently it is to be interpreted as a complex spiral, with paired traces one more apical than the other, and each of the pair given off from a separate primary bundle.

Secondary wood

The zone of secondary wood has a maximum radial thickness of 2.8 cm. and a minimum of 1.5 cm., an unknown amount having been lost previous or subsequent to fossilization.

Six concentric zones are visible in sections (fig. 15), and extend in a uniform manner about the stem. They are growth zones caused by the alternation of wider (spring wood) and narrower (summer wood) elements. As indicated in figure 9, the transition from summer to spring wood is definite but not always abrupt. The larger tracheids include about eight to ten cells in radial series. Their average radial dimension (60 μ) is about twice that of the smaller tracheids. There is a gradual transition from larger to smaller elements. The zonation appears more distinctly under a hand lens than when observed under higher magnification. This is due to the absence of inclusions in the larger tracheids, whereas those formed later are commonly filled with dark materials which may be interpreted as stored substances or as organic matter derived from the thick walls, which broke down in part during the process of infiltration. The



FIGS. 11–19.—Fig. 11, transection of emerging trace showing mesarch arrangement and a few parenchyma cells included in bundle, also tendency for centrifugal metaxylem to be radially grouped; ×52. Fig. 12, mesarch bundle in submarginal position; outline is elliptical with longest dimension in radial plane; ×56. Fig. 13, radial section of secondary wood with crowded multiseriate pits uniformly distributed; ×59. Fig. 14, general view of stem as found; ×¼. Fig. 15, transverse cut of stem showing large pith and growth zones; ×⅜. Fig. 16, transection of secondary wood at pith border showing slight dilation of rays which are at most three cells wide; ×52. Fig. 17, tangential section of secondary wood about 1 cm. from pith, showing rays narrow and high; ×44. Fig. 18, radial view of bordered pits with slitlike pores; ×235. Fig. 19, tangential section of secondary wood and rays cut close to pith border; ray cells approximately isodiametric; ×52.

zonation is less sharply defined than in modern conifers, but is more definite than in most Paleozoic woods thus far described. The first zone is about 3 mm. wide, the second 7 mm., the third 3 mm., and subsequent zones average approximately 2 mm. in width.

Neighboring wood rays, as seen in transverse section, are separated by four or five radial rows of tracheids which average 29 μ in tangential and 32.5 μ in radial diameter. They are roughly rectangular, with the corners rounded.

Wood rays are dilated slightly where they meet the pith (figs. 16, 19), owing in part to the ray cells being wider in this region, and also to the frequent occurrence of rays that are three cells in width. Within 1 mm. of the pith, the wider rays are narrow, commonly uniseriate, and their cells are radially elongated. Except near the pith margin, rays are typically uniseriate or biseriate in part, and often of great height. Rays thirty to fifty cells high are common and occasional ones exceed ninety cells in height (fig. 17). The ray cells where uniseriate average about 40 μ high and 30 μ wide, except near the pith where they are approximately 70 μ in each dimension (fig. 19). Well out in the secondary wood where the ray cells are multiform, they have transverse or sloping end walls, and average about 125 μ in radial length. No ray tracheids have been found.

On tracheids of the secondary wood, pits are typically limited to the radial walls, are uniformly distributed, crowded and hexagonal, or occasionally rounded and contiguous (fig. 13). They are most frequently arranged in three vertical series and alternately grouped. The pore is an inclined slit (fig. 18). Tangential pits occur sparingly on tracheids abaxial to an outgoing trace. The protoxylem bears spiral markings, and the metaxylem of the submarginal bundles is mostly transitional between reticulate and pitted. True pits could not be found in the metaxylem except in the outgoing trace where they occur on both radial and tangential walls. The transition from spiral elements to pitted in the primary wood of the trace takes place much more quickly than is characteristic of *Cordaites* (**9**).

Relationship

In many structural features this stem resembles *Pitys*, and more particularly *P. withami* (**8**) from the Lower Carboniferous of Scotland. It has in common with *Pitys* a large number of mesarch

primary bundles buried in the pith near its margin. These bundles are small, of fairly uniform size, and turn out to the secondary wood zone only where they are about to connect with outgoing leaf traces. Other structural resemblance to *Pitys* is found in the large continuous pith; the prominent development of secondary wood; the formation of growth rings; parenchyma elements included in the outgoing traces; and metaxylem which is pitted in the trace but prevailingly scalariform or transitional between reticulate and pitted in the submarginal bundles.

There are, however, several structural features which differ from those of *Pitys* and required the establishment of a new genus. The most obvious of these are: restriction of primary bundles to a submarginal zone; compact wood with uniseriate rays; paired leaf trace arrangement; position of reparatory strands; and the cytology of the pith.

In all species of *Pitys* so far as known there are in addition to the submarginal ring of bundles many others which in transverse sections appear scattered throughout the pith column (**7**). The primary bundles of this stem are all submarginal in position, but they lie somewhat deeper in the pith than corresponding bundles of *Pitys*. Six to thirteen cells may intervene between them and the margin whereas in *Pitys* two to three cells commonly separate them from the secondary wood. Certain of the primary bundles are smaller and more deeply imbedded than the others. At two or three places in the stem radial division of bundles is observed. Such division may be interpreted as a simplified pityean arrangement. Other bundles divide in a diagonal or tangential direction, however, and they may all be instances of lateral connections between neighboring strands.

The different species of *Pitys* are determined largely by relative width of the wood rays. Throughout the genus they are conspicuous, being widest in *P. rotunda* (**7**) and narrowest in *P. withami* (**7**). In the latter they may be six cells in width although most of them are biseriate. In this stem both primary and secondary rays are uniseriate or biseriate in part. In this respect it has a density similar to that of *Cordaites*. There is, however, this interesting transitional feature—the presence of rays of great height.

A paired leaf trace arrangement has not been described for any of the Pityeae although it is a common feature of Paleozoic seed plants.

It is typical of the Lyginopterideae and the more herbaceous Calamopityeae, both of which bear certain structural resemblance to this specimen. Paired traces also occur in the Poroxyleae and Cordaiteae of the Cordaitales. In the several species of *Pitys* (**5, 6, 7**) and *Callixylon* (**2**), and in *Archaeopitys* (**11, 12**), the traces—so far as they have been observed—pass through the wood singly, and in *P. dayi* (**7**) are known to divide external to the secondary wood. The occurrence of paired traces in wood of this stem thus tends to set it apart from the genus *Pitys*.

The manner in which reparatory strands are formed is almost identical with the condition described for *Callixylon erianum* by ARNOLD (**1**), but differs considerably from *Pitys* in which there is no reparatory bundle directly opposite the trace, continuity being maintained through lateral connections with adjoining primary bundles (**7**).

In all species of *Pitys* so far described the pith appears to be composed of three types of cells (**7**), scattered secretory elements, storage cells, and thin-walled parenchyma. The latter for the most part surrounds the primary strands. The pith of this stem is composed almost entirely of thin-walled parenchyma which is radially elongated. None of the secretory type have been observed, and storage cells occur sparingly near the pith margin.

Certain features of this stem resemble *Callixylon* more closely than *Pitys*. As indicated in a preceding paragraph, this is true of the reparatory strands. The restriction of primary bundles to the submarginal zone may be considered intermediate between *Pitys* and *Callixylon*, for in the latter they are grouped in a ring about the pith margin, some imbedded and others in contact with secondary wood. The dense wood with narrow rays resembles that of *Callixylon whiteanum* (**3**). In contrast with *Callixylon*, tracheids are absent from wood rays and pith, there is uniform radial pitting, and the leaf traces are paired.

This stem is from the same horizon as *Pycnoxylon* (**5**) and bears certain resemblance to it, particularly in the dense wood, large pith, and a paired trace arrangement. *Pycnoxylon* differs in having primary bundles endarch below the nodes, tracheids in some of the rays, reparatory strands lateral to the leaf trace, traces which pass out

forming a wide angle with the stem axis, primary bundles more closely associated with secondary wood, and paired traces that originate from the same strand.

Recently the writer has described another stem from the Reed Springs Formation (**6**). It is similar in certain respects to this specimen but because of features which indicate affinity with pteridosperm stock should be taken into consideration. It has in common with this stem a large pith (52 mm. diameter), one hundred or more submarginal bundles, and paired traces.

It further resembles *Pitys* in having wide rays, primary strands with lateral connections, and traces that pass out through the wood forming a wide angle with the stem axis. Affinity with certain Calamopityeae is indicated in the reduction of primary bundles below the nodes, an endarch tendency, sclerotic clusters in the pith, paired traces, and the evidence that leaves were large. Certain of these features may be interpreted as indicating either relationship or parallel development; for example, sclerotic clusters occur in the pith of *Eristophyton* and *Lyginopteris* but have not been reported as occurring in the pith of any of the Pityeae. Since they are present in the cortex of *P. dayi*, they may readily have been more extensively distributed in other members of the family. In similar manner the reduced state of the primary bundles with the attendant irregularity of protoxylem position may be interpreted as an adaptive variation associated with numerous bundles and very short internodes.

The occasional groups of traces which characterize this specimen probably connected with a fruiting branch of cauline origin. The manner in which reproductive structures were borne has not been determined for *Pitys* or *Callixylon*. In *Poroxylon* (**9**) adaxial buds which may have formed a fruiting branch have vascular connections with the two neighboring bundles, one in the anodic and the other in the kathodic position in relation to the traces.

Although the stem described in this paper shows certain affinity with pteridosperm stock, the very large pith, many primary strands, absence of pitted elements in the metaxylem of the bundles, and the arborescent tendency all indicate a closer affinity with the Pityeae than with any known members of the Lyginopterideae or Calamopityeae, with which there is a similarity in general organization.

Until a more extensive comparative study is completed on the Reed Springs flora, it seems advisable to place the stem tentatively in the Pityeae as a new genus and species for which the name *Megalomyelon myriodesmon* is proposed. The generic name refers to the large continuous pith, and the specific name to the very large number of small submarginal primary bundles.

Megalomyelon myriodesmon gen. et sp. nov.

Pith 50 mm. wide, continuous, without ducts or sclerotic nests, cells elongated radially. Primary bundles, about 100 in number, mesarch, submarginal in position, becoming marginal only near a node, branch sparingly, not confluent, follow vertical course, protoxylem spiral, metaxylem transitional between reticulate and pitted. Reparatory strand single, smaller than leaf trace, mesarch, on same radial plane as outgoing trace. Leaf traces paired, one more apical than the other, derived from separate bundles, mesarch, not greatly enlarged at node, small amount of parenchyma included, metaxylem radially aligned centrifugally, slightly larger centripetally, pitted on all walls. Parenchyma embayment at pith margin continues as crescent over outgoing trace. Secondary wood of trace limited to abaxial side. Secondary wood abundant, with growth zones; spring wood average 60 μ radial diameter, summer wood 32.5 μ. Wood slightly distorted abaxial to outgoing trace, closes over trace terminus quickly with slight distortion. Wood rays at pith border one to three cells wide, approximately isodiametric, and prevailingly high (maximum ninety cells). Wood rays 1 cm. from pith uniseriate or biseriate in part, high (ninety cells maximum), muriform, about 125 μ radial dimension, transverse or sloping end walls, ray tracheids not observed. Pits prevailingly crowded, hexagonal, uniformly distributed, and commonly in three alternate series, occasionally rounded and contiguous. Pore an inclined slit, fully bordered. Leaf trace sequence interrupted by occasional clusters of traces.

Summary

1. The petrified stem the structure of which is here described came from the Reed Springs Formation of the Mississippian. It is infiltrated with siliceous materials and probably represents a portion of the main trunk. Since the cortical structures are lost and an unknown amount of the secondary wood has eroded, the maximum

diameter of 9.5 cm. indicates that the specimen represents a plant which originally had the dimensions of a small tree.

2. There is general agreement in most structural features with the genus *Pitys*, with which it is more closely related than with any other known form. The most significant points wherein it differs from *Pitys*, namely, paired leaf traces and narrow rays, are characteristic of Cordaites on the one hand and of certain Calamopityeae on the other. The leaf trace arrangement is so unlike any known member of the genus *Pitys* that the stem is given generic rank, but because of the prevalence of pityean features is included in that family.

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DETERIORATION OF GRASSLAND FROM STABILITY TO DENUDATION WITH DECREASE IN SOIL MOISTURE[1]

J. E. WEAVER AND F. W. ALBERTSON

(WITH SEVENTEEN FIGURES)

Introduction

The great drought has now prevailed intermittently in the midwest for a period of seven years. Damage to native vegetation in 1934 has been partially repaired, but further destruction has also occurred. Continued study of the shiftings in dominance among the grasses has revealed widespread losses of certain species and partial or complete replacement by others. Forbs also have greatly diminished, both in number of species and in abundance, and changes in structure of vegetation generally have been pronounced (**3**, **4**). Hence it seemed advisable during 1939 to study the degree of deterioration of the grasslands, if any, from western Iowa, through eastern Nebraska and Kansas, to western Kansas where in places almost complete destruction of vegetation has occurred. This study included monthly determinations of water content of soil to the depth of root penetration of the grasses, and other major environmental factors characterizing the growing season. The rate of growth of vegetation and density of cover were determined, as well as the distribution of forbs.

Groups of prairies whose previous history was known were selected for study (fig. 1). Soils were all similar, being of silt-loam texture; although those at Lincoln, Nebraska, and eastward belong to the Prairie zonal group, while those under lighter precipitation westward are Chernozems. All are of high fertility, and water is the chief limiting factor to plant growth.

[1] Contribution no. 121 from the Department of Botany, University of Nebraska. This investigation was aided by a grant to the senior author from the Penrose fund of The American Philosophical Society.

Environmental relations

The critical environmental factors characteristic of the drought are low unevenly distributed precipitation, deficiency of soil moisture and accompanying high temperatures, high evaporation rates, and increased wind movement.

FIG. 1.—Map showing the four groups of stations where studies were made in 1939. Iowa group and Lincoln-Hebron-Nelson-Belleville group were dominated by *Andropogon scoparius* and *A. furcatus*. *Agropyron smithii* was dominant at Clay Center–Carleton–Montrose group, and *Bouteloua gracilis* and *Buchloe dactyloides* at the three most southwesterly stations.

PRECIPITATION AND WATER CONTENT OF SOIL

Stations for water-content sampling were selected with reference to typical and relatively uniform composition of vegetation. In each group of stations were included various gentle slopes and nearly level uplands, where water content of soil depended entirely upon the precipitation in that area. Samples were secured regularly near the middle or latter part of each month from April to August, inclusive.

At the five stations in Iowa, where the mean annual precipitation ranges from 29.5 to 33.7 inches, rainfall during the several months

was sufficient to keep both soil and subsoil constantly moist to a depth of 6 feet at least (fig. 2). In April, at all stations the surface 24 inches of soil had 10–20 per cent or more moisture available for growth, and the deeper soil 2–10 per cent or more.[2] During the entire growing season, soil moisture depleted by the growing vegetation was restored more or less regularly and completely. To a depth of 24 inches, the available supply ranged continuously between 2

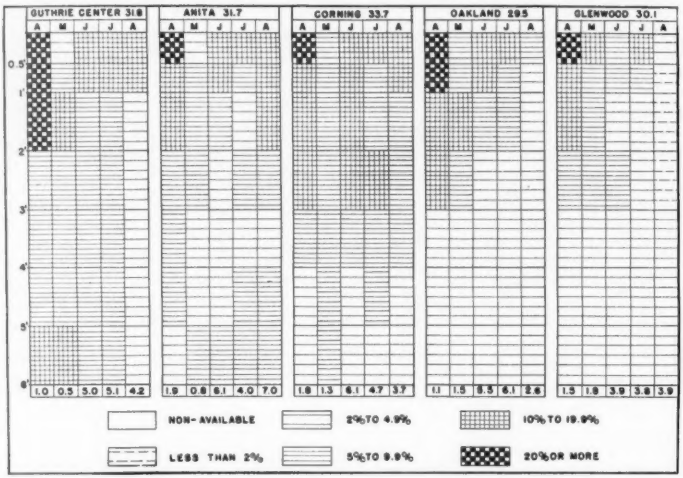


FIG. 2.—Available soil moisture to depth of 6 feet at the five Iowa stations during 1939. Mean annual precipitation at each station follows the name, and total current monthly rainfall is given at the foot of each column.

(but usually 5) and 10 per cent or more. A single exception occurred at the most westerly station (Glenwood), where in August water content was reduced to a minimum of less than 2 per cent. Water in the deeper soil was also always available, usually in the amount of 2–5 per cent or more. This moderate water content of soil resulted not only from the rather regular and well distributed rainfall, but also from its ready entrance into these silt-loam soils, which were kept in a porous, water-receptive condition by the continuous cover of grass.

[2] Total water content of soil minus the hygroscopic coefficient (which was determined for each soil depth at each station) is designated as water available for growth. The hygroscopic coefficients, with few exceptions, ranged between 9.1 and 12.9 per cent.

The mean annual rainfall at the second group of stations varies from 26.2 to 27.9 inches. Precipitation is sufficient, if well distributed, to keep the soil continuously moist under a cover of grassland, but this was a season of drought. Rainfall of June alone was equal to (or slightly below) the normal at all stations in this group (fig. 3). Early spring sampling showed an available water supply of 2–20 per cent or more in the surface 24 inches, except at the Kansas station

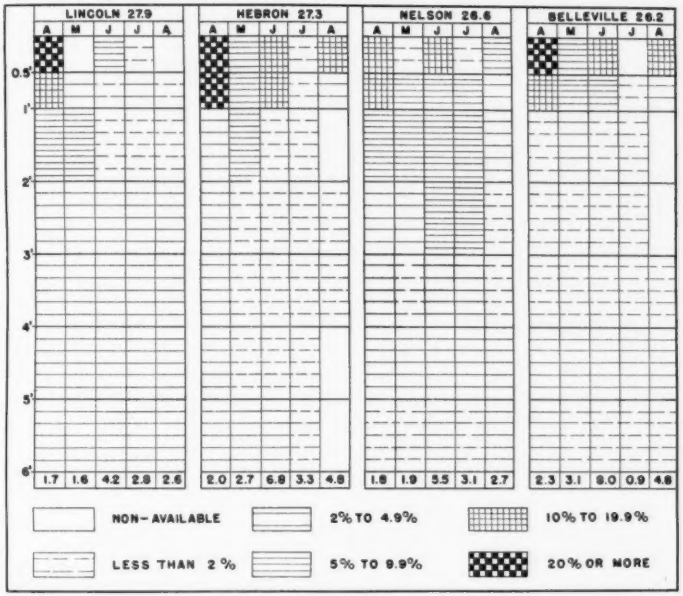


FIG. 3.—Available water content of soil and mean annual and current monthly precipitation at Nebraska-Kansas bluestem stations in 1939.

(Belleville) where the second 12-inch level had less than 2 per cent. The subsoil to 6 feet had consistently 2 per cent or more of available water, except at three levels at two stations where it was less than 2 per cent (fig. 3). The surface 24 inches maintained a good water content continuously at only one station (Nelson); at the others it was reduced to the point of nonavailability at some level one or more times, and at several samplings to less than 2 per cent. In the subsoil at Lincoln a small supply (2–4.9 per cent) was continuously available to 6 feet; at the other stations available water was fre-

quently reduced to less than 2 per cent, and at some periods none was available at certain levels.

While reduction in water content was brought about by a considerably depleted plant cover, much bare ground was also present. This decreased the efficiency of absorption of water to a considerable degree and promoted greater loss by runoff. Very numerous experiments, at practically all of the stations in the second and third groups, showed that within the same square meter water penetrated one and a half to two or even three times as rapidly where grasses still clothed the soil as where the ground was bare.

The three stations, Clay Center, Carleton, and Montrose, form a natural grouping, despite the fact that the mean annual precipitation at Carleton is slightly above that of two in the preceding group (fig. 4). The dominant vegetation at these stations is western wheat grass (*Agropyron smithii*) and not bluestems (*Andropogon scoparius* and *A. furcatus*), as at all the preceding prairies. Hence an important factor affecting the early exhaustion of soil moisture was, unlike that of bluestems, the very early and rapid growth of the wheat grass.

A good available water content occurred in the surface foot in April (10 to more than 20 per cent). Available water was sufficiently replenished at Carleton so that at least very small amounts continuously occurred in the first foot, but at the other stations complete exhaustion over long periods was repeatedly found. At 1–2 feet in depth, small amounts of water were available except in August at Carleton, but moisture was never available at this level at Montrose. Below 2 feet, no water was available for growth at any level of sampling after April, except less than 2 per cent at 5–6 feet depth at Carleton and similar small amounts (or none) at Clay Center.

These prairies had not only suffered greater losses of plant cover by previous drought than the preceding group, but also a rather complete change in plant population. This resulted in part from a cover of dust of variable thickness which clogged the soil pores so effectively that much of the torrential rainfall never entered the soil but was lost as runoff. Moreover, it has been found that wheat grass furnishes only poor protection to soil from surface puddling

when water falls upon it. Compared with the rate of penetration in adjacent, small, relict areas of bluestems, the period required for the entrance of similar amounts of water (as in light to moderate showers) is one and a half to two times as great.

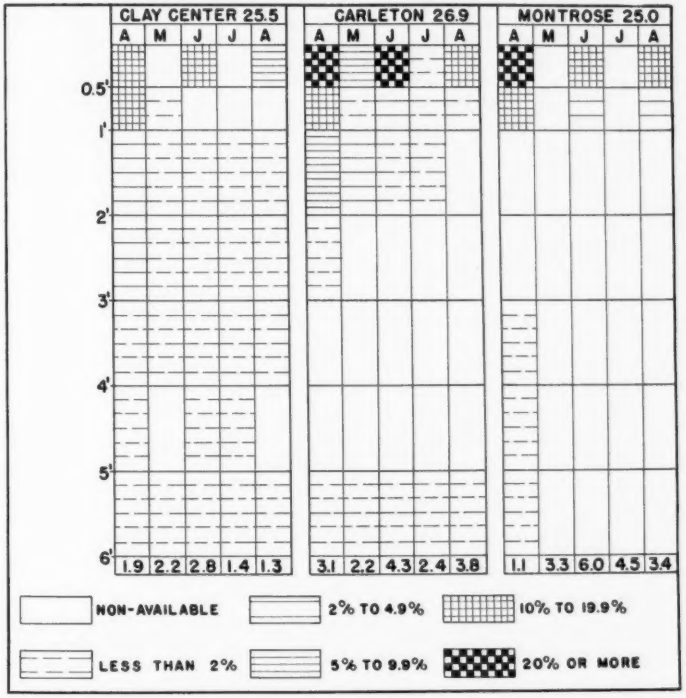


FIG. 4.—Mean annual precipitation, current monthly precipitation, and available soil moisture at Nebraska-Kansas wheat-grass stations in 1939.

The last group of stations has a decidedly lower mean annual precipitation, 18.9–23.3 inches, than any of the preceding (fig. 5). The mixed prairie grassland has here been more or less completely reduced, either by the recent drought (Phillipsburg) or by long continued grazing and drought (Hays and Dighton), to a short-grass plains disclimax. On each prairie enough silt has been deposited as dust to seal the soil so effectively that runoff has been greatly in-

creased. It was further accelerated by the small amount of vegetative cover and consequent lack of much obstruction to water movement. Thus, despite the rather high rainfall of June, the surface soil alone was moistened. Usually in this more arid region much of the precipitation is of low efficiency, because it falls either as torrential showers with high runoff or in many small showers followed by

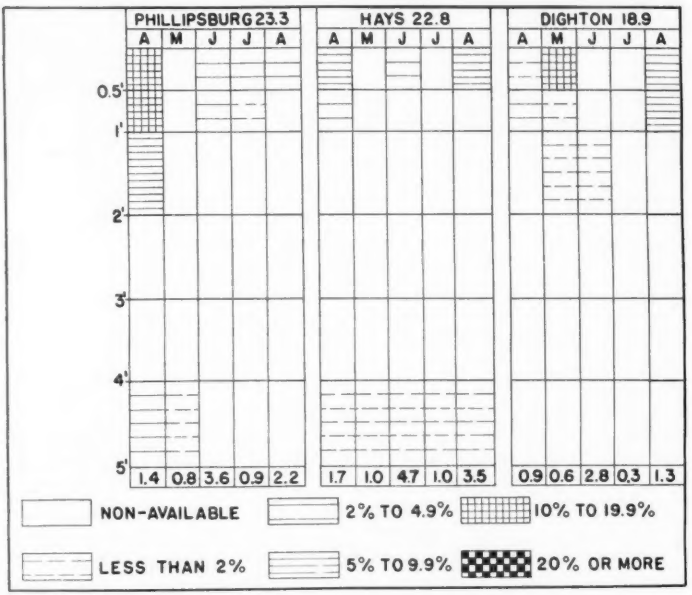


FIG. 5.—Chart showing relatively small amounts of water available for growth of vegetation in western Kansas and lack of subsoil moisture in 1939.

bright sunshine and often by high winds; hence the water is soon lost from the soil by evaporation. For example, the 4.7 inches of rain in June at Hays fell on thirteen different days. Only four of the rains exceeded 0.3 inch in amount and the heaviest for any day was 1.5 inches.

The surface foot of soil was moist (mostly from melting snow) in April, but only slightly so at the most westerly (Dighton) station. At various times throughout the summer no surface soil moisture was available for growth. The deeper soil, with rare exception, had

no available water. There was no reserve, even in early spring. At Hays it seems that a small amount of residual moisture remained unused at the 5-foot depth.

The charts present an accurate general picture of water content of soil. Weekly determinations add but little information to the general sequence. At Hays, for example, weekly samplings showed that during the first three days of May small amounts of water were available in the surface 6 inches. Water was also available the first five days of July and unavailable the last four days in August. At the second 6-inch level it was available in small amounts in the first week of May. Otherwise the weekly data conform entirely with the chart.

Thus while the grasslands of Iowa had an abundant to moderate but continuous supply of available soil water, those in the second group had—after early spring—only a low supply and were repeatedly threatened with drought. At the third lot of stations, available subsoil moisture was the exception; at Carleton the surface foot was usually moist, but complete exhaustion occurred repeatedly in the other prairies. Water was available only in the upper soil at the most westerly stations and only at two or three periods during the growing season.

Temperature, evaporation, and wind

Mean temperatures for April were practically normal, and only 2.7° F. (or less) higher in the most southerly group of stations (53.7°) than in the eastern or two central ones. They varied less than 1° among groups during May, but at all stations they were about 5°–8° higher (68° F.) than normal. A difference of only 2.5° between the lowest and highest average temperatures occurred in June, the highest average (75.2°) being recorded in west-central Kansas. At all stations, temperatures were only 2°–4° above normal. During July, temperatures from Iowa to western Kansas ranged from 77.9° through 82.8° to 84.0°. They were approximately 3°–6° higher than normal, the increase being slightly the greatest in western Kansas. August was cooler, mean average temperatures being 72.0°, 75.6°, and 77.9° in the preceding sequence. Variation at any station above or below the normal did not exceed 2°.

Maximum temperatures during periods of stress were neither so

high nor of so great duration as during 1934 or 1936. The writers believe that high temperatures in grasslands have not been the direct cause of death of plants, but that they are merely one of several factors which intensified drought. Death due directly to drought resulted from lack of water. Where a water supply was artificially maintained for native species, no injury has been found.

Water loss as measured by rate of evaporation was much greater at the westerly stations than at those eastward. During April and May it was 39–51 per cent higher at Hays than at Lincoln. In June and July it was about twice as great at Hays, the difference in rate decreasing to 63 per cent in August. The lowest loss at Hays was 8.9 inches from a free water surface in April; the highest 20.6 inches in July.[3]

Comparison of the average daily losses from May to August inclusive during the extremely dry, hot summer of 1934 and that of 1939 is pertinent. These were measured by white, spherical, porous cup atmometers. Evaporation in 1934 was 82 cc., in 1939 only 49 cc. During the hottest month of 1939 (July) the average daily loss was 48 cc., compared with 96 cc. in 1934. Compared with evaporation losses during 1933 (just preceding the severest year of drought), weekly averages were higher only three times. During the period of greatest stress (third week of July), however, losses were 88 cc., twice as great as during the same period in 1933.

Wind movement increases westward. During April, 1939, it was about twice as great at Hays as at Lincoln. In May and June it was about three times as great, in July three and a half times, and in August there was more than three times as much wind movement westward. From April to August inclusive it totaled over 28,000 miles at Hays, at Lincoln about 10,000.[3] Wind is increasingly detrimental to vegetation westward, not only because of the damage done by wind-carried dust but also because of frequent high velocities immediately following showers. This increases both evaporation and transpiration, which in turn accelerate the depletion of soil moisture.

While neither wind movement nor evaporation losses were as great as during certain other drought years, at most stations at

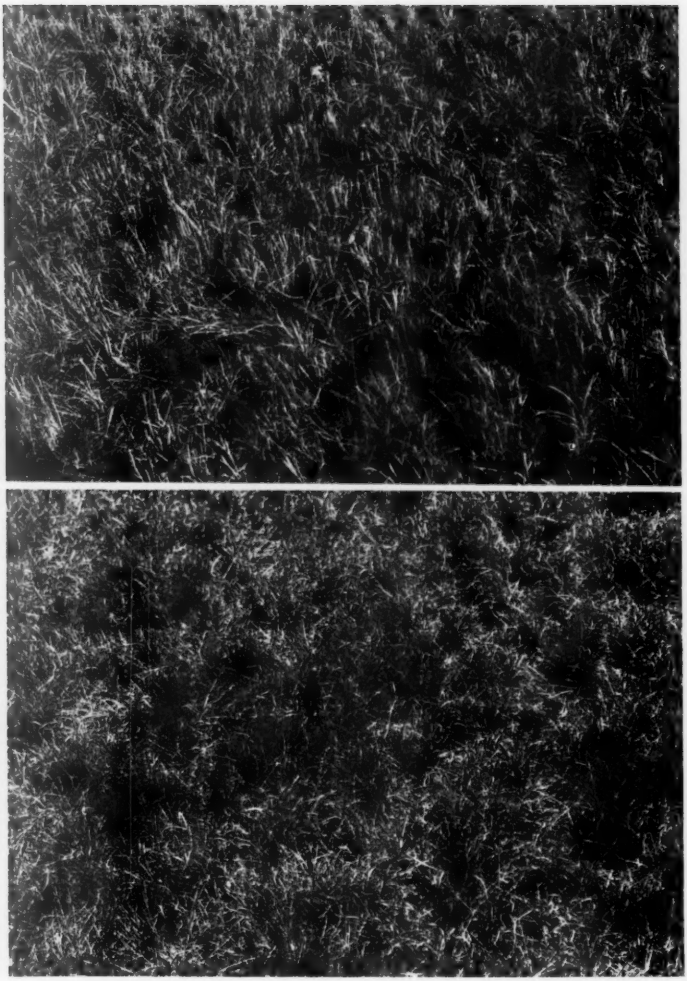
[3] Data from U.S. Department of Agriculture, Weather Bureau.

least they were considerably greater than normal during the pre-drought period. Loss of vegetative cover increased wind movement near the soil and greatly accelerated direct loss of soil moisture by evaporation. Had the soil provided ample water for absorption, however, it seems certain that vegetation would have suffered little, even under these conditions of somewhat increased temperatures, greater stress of evaporation, and increased wind movement.

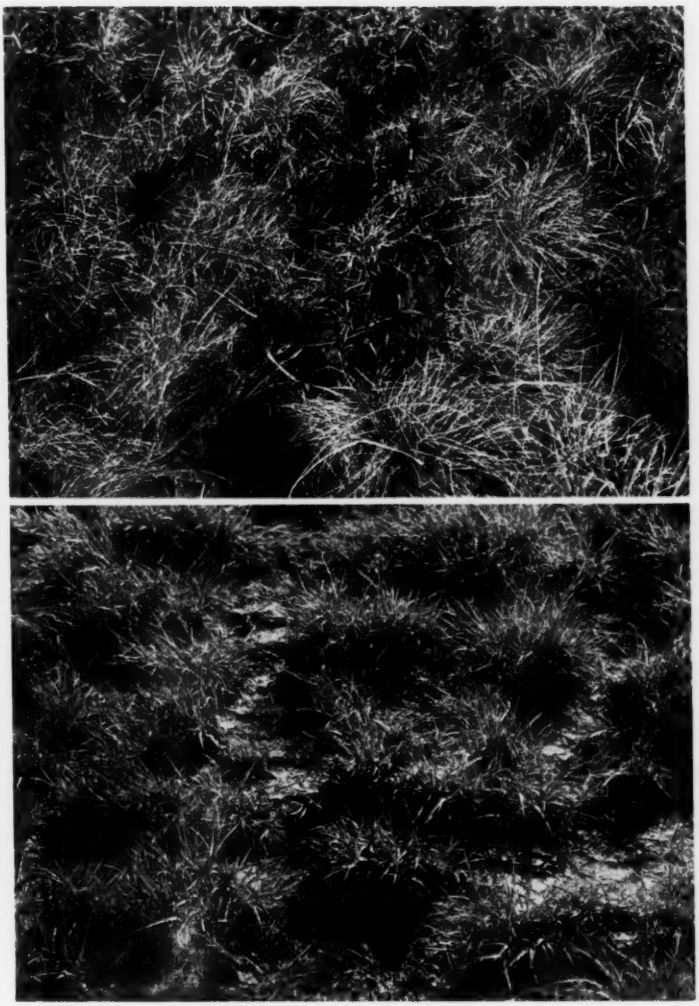
Previous deterioration of plant cover

The Iowa prairies had not been affected as regards change of structure by the great drought of 1934 or subsequently. An exception was some death on the drier slopes at Corning, but complete recovery had occurred by 1939 (**3**). In spring, following the preceding mowing in fall, the soil at all the stations was completely obscured by stem bases and a continuous cover of fallen debris a few millimeters in thickness. There was no bare ground (figs. 6, 7). Numerous lichens and mosses were found on the damp spongy earth between the tufts of sod. By midsummer the tall dense cover of vegetation, with a wonderfully developed understory and an abundance of forbs overtopping the grasses, revealed the prairies as of old (**5**). At no time during the entire growing season was plant development retarded by drought.

Deterioration of the prairies of Nebraska during the drought of 1934 and the results of the drought in both Nebraska and Kansas during the following year have been described (**6, 3**). With successive dry years further deterioration had occurred (**4**). At the second group of stations in the spring of 1939, much soil was bare. Soil-sampling stations were selected in the more stable portions of each of the four prairies where the cover of bluestems, although greatly thinned, remained intact. A chief change was an increase of other grasses, especially *Bouteloua curtipendula*. Widely spaced bunches and tufts of grasses with much bare soil, almost without protecting debris, were the characteristic features (figs. 8, 9). The basal cover had been reduced to two-thirds to one-half its former area. The ground layer of low-growing species and of lichens and mosses in spring had practically vanished. The remaining vegetation produced much less debris. The lack of moisture and shade caused it to



FIGS. 6, 7.—Fig. 6 (above), basal cover at Guthrie Center, Iowa, late in April, 1939. The bluestems, etc., were mowed late in fall of preceding year, yet the leafy stem bases and fallen debris completely conceal the soil. Fig. 7 (below), similar view at Oakland, Iowa, April 21. Note complete cover of dormant grasses and leaf mulch.



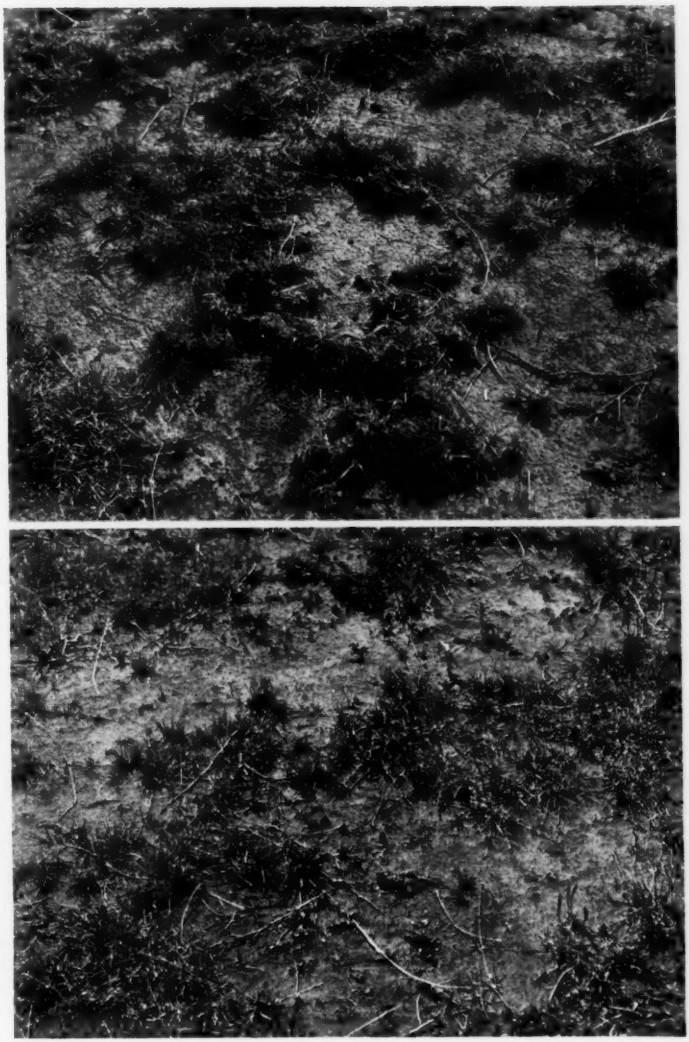
FIGS. 8, 9.—Fig. 8 (above), basal cover of little bluestem and other grasses which had made some fall growth after early mowing in autumn of 1938. Soil between bunches of grass is almost bare. Photographed Lincoln, Nebraska, April 29, 1939. Fig. 9 (below), similar view at Nelson on June 16, 1939. Widely spaced bunches and bared soil permit much loss of precipitation by runoff.

shrivel and dry, and high winds carried it away, leaving the ground quite bare. Each successive year of drought had further reduced a once abundant growth of forbs, until the grasses almost alone constituted the mid-layer, which was overtopped sparingly by small remnants of the once abundant taller forbs.

Great modifications, involving an almost complete change in the species of dominant grasses, had occurred at the three more xeric stations—Clay Center, Carleton, and Montrose. The bluestems had been all but exterminated by drought and deposits of dust, the Carleton prairie being least affected by dust burial. A wave of *Agropyron smithii* had swept over these prairies, taking rather complete possession, but this grass was being partly replaced by rapid spread of *Bouteloua curtipendula* (2). Sufficient moisture had not been available for the sod-forming wheat grass to aggregate densely. Between the widely spaced stems much unoccupied soil occurred, other plants had nearly all died, litter was scarce, and bare ground formed a continuous pattern everywhere.

Drought and dust had caused greater deterioration at the western Kansas stations than in any other group. The ungrazed prairie at Phillipsburg had lost nearly all its mid grasses (except *B. curtipendula*) and was largely converted into an open stand of the short grasses—*B. gracilis* and *Buchloe dactyloides* (fig. 10). Subsequently side-oats grama (*Bouteloua curtipendula*) had increased greatly. Only a single layer of vegetation remained, however, since these grasses alternated and did not intermix, while forbs occurred only sparingly. Where *B. curtipendula* grew, only 10 per cent of the soil was occupied, the wide spacing being an adaptation to drought. The patches of short grasses were so widely spaced that they probably occupied only about 10 per cent of the total of the remaining surface, since many irregular areas of a few square feet to a few square yards were entirely bare.

The two short grasses in the moderately grazed range at Hays were formerly intermixed almost equally to form a basal cover of approximately 85 per cent (1). This had been reduced by a light cover of dust and drought to only 16 per cent (fig. 11). In 1939, blue grama (*Bouteloua gracilis*) formed three-fourths of the vegetation, buffalo grass (*Buchloe dactyloides*) having suffered the greater losses.



FIGS. 10, 11.—Views of short grasses showing conditions of vegetation at most westerly group of stations early in June, 1939. Fig. 10 (above), nearly pure *Bouteloua gracilis* in lightly grazed range representative of this type at Phillipsburg, Kansas. Fig. 11 (below), *Buchloe dactyloides* in well kept pasture, illustrative of depletion of cover near Hays, Kansas.

Little bluestem (*Andropogon scoparius*), other mid grasses, and most of the forbs had succumbed to drought.

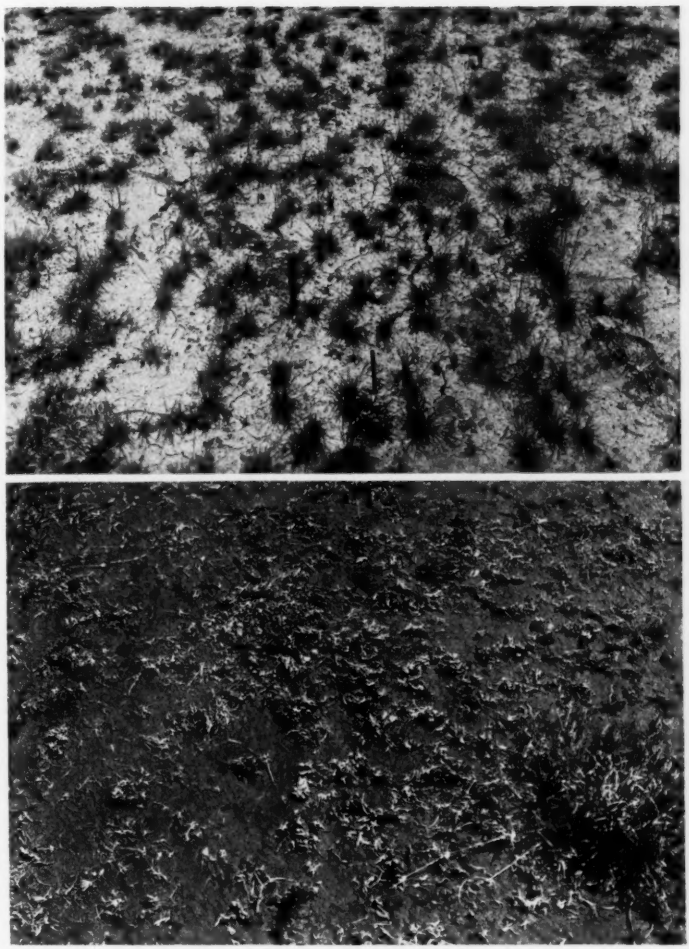
Blue grama and buffalo grass, in approximately the proportions of 3:1, constituted practically the entire cover of 12–15 per cent at Dighton. This prairie was also grazed, as are practically all the upland midwestern grasslands, since the forage is usually too short to cut for hay. At all three stations, plants of the lower layer had all but disappeared, and any debris from the scanty vegetation was swept away by high winds, leaving the surface nearly bare (figs. 12, 13).

Thus the preceding years of deficient moisture, great dust storms, and other unfavorable environmental conditions, while scarcely extending to the prairies of Iowa, had an increasingly deleterious effect toward the central area of greatest damage in western Kansas.

Plant development in relation to drought

Growth in spring was delayed at the Iowa stations because of late heavy snow. For most species it began late in April and progressed steadily, uninterrupted by drought at any time during the summer. A sprinkling of 2–5 per cent of *Poa pratensis* throughout and its blossoming at a height of 10–12 inches in May were evidence of the mesic conditions that prevailed. Height growth of the foliage of bluestem grasses was complete late in July, when a level of 15–20 inches was attained; after this flower stalks began to develop. The yield of forage was about normal, one to one and a half tons per acre.

Early spring growth was about normal at the second group of bluestem stations. The prairie vegetation was much less luxuriant because of the severe thinning of the grasses and the fact that the bunches were poorly filled with stems, both effects of the preceding drought. In Iowa, for example, a given basal area of little bluestem was 75–90 per cent occupied with leafy stems, but in this group the space was usually only one-third to one-half filled. Moreover the understory was represented by mere fragments or none of the usually widely distributed *Viola pedatifida*, *Antennaria campestris*, *Astragalus crassicarpus*, *Sisyrinchium angustifolium*, and the rosettes of *Hieracium longipilum*, *Erigeron ramosus*, and others. Precipita-



FIGS. 12, 13.—Fig. 12 (above), typical ground cover at Dighton, Kansas, August, 1939, on lightly grazed range. *Bouteloua gracilis* and *Buchloe dactyloides* constituted nearly all the scanty vegetation, but *Malvastrum coccineum* and a few other xeric forbs occurred. Eighty-eight per cent of soil is bare. Fig. 13 (below), almost complete loss of vegetation by burial with dust, which wind has now swept away, leaving dead bases of short grasses as evidence of formerly good cover. Photographed Sharon Springs, Kansas, June 2, 1939.

tion in May was deficient. Grasses of early growth, especially *Stipa spartea* and *Agropyron smithii*, soon exhausted their water supply, and late in the month were wilted and the leaves much discolored. *Stipa* produced very little seed, and flower stalks of *Koeleria cristata* were short. Abundant rainfall in June permitted normal development of the vegetation. Drought prevailed at several periods in July, with temporary revival of growth following showers. By mid-July both andropogons were wilting at most stations and many leaves were dead and brown. Pronounced drought injury was evident during August. The grasses dried more or less completely, after producing the reddish or brownish colors of late autumn, although some new greening of basal leaves occurred following showers in September. Flower stalks were not produced by the bluestems, except at Nelson, which had the best water supply (fig. 3). The formerly pronounced seasonal aspects were poor in spring and early summer, and there were none thereafter. Yield of forage was light, only one-third to one-half ton per acre.

Growth at the third group of stations (Clay Center–Carleton–Montrose) began about a month earlier than at the preceding one, since the controlling species is the early starting *Agropyron smithii*. Its dominance followed the death, by drought and (in part) burial by dust, of the bluestems. Loss of the bluestems was accompanied by death of a considerable portion of the population of forbs and other grasses. As a result of the lighter rainfall and the earlier absorption of soil moisture, growth was poor even in early summer. The spring aspect was poorly developed and lack of seasonal aspects characterized the remainder of the year. The wheat grass was 5 inches tall by April 12, at a time when the prairies were dry and the old foliage sprinkled with dust. Drought in May caused the leaves to roll tightly, and growth temporarily ceased. During June, when blossoming normally occurs, the foliage level was only 7–14 inches high and flower stalks were few or none, except at Montrose where a scanty crop was developed. During the remainder of the summer, the stems and leaves were dry and the intermixed side-oats grama—and even the blue grama—failed to produce flower stalks. No plant was in blossom; indeed the entire landscape presented no green color. An exception occurred at Carleton, where a good crop

of short flower stalks was produced by side-oats grama in July. At Montrose, a prairie fire burned clean everything above ground over half the area the first week in July. Of the few scattered forbs, all but those of spring failed to flower; grasses developed so poorly that the crop of hay was too scanty to warrant cutting.

Growth occurred only intermittently at the short-grass stations, since there was no reserve moisture and vegetation depended entirely upon the light current precipitation. A few vernal species with large storage organs, especially *Anemone caroliniana* and *Allium nuttallii*, grew in response to moisture furnished by melting snow, as did certain annuals, notably *Plantago spinulosa*, *P. purshii*, *Lepidium densiflorum*, and *Hordeum pusillum*. Few annuals, including *Salsola pestifer*, developed beyond the seedling stage or attained a height of more than 1–3 inches. Even the perennials with food reserves usually failed to produce flower stalks; only a few windflowers, for example, partly unfolded their blossoms on dwarfed stems which dried with the exhaustion of available water.

Rains of late May or early June promoted ephemeral growth of the dusty, dry short grasses, which was soon terminated by midsummer drought. The prairies were sere and brown until once again the surface soil was moist in August. Then stolons of buffalo grass spread laterally 5–10 inches before the moisture was exhausted, the roots at the nodes becoming successively shorter and more subject to death from drying as the distance from the parent plant increased. Where some protection was afforded from too rapid drying, as about the bunches of cacti, blue grama grass put forth flower stalks and blossomed in late fall. But later examination revealed that few viable seeds were matured. The dwarfed, most xeric forbs grew precariously, with little or no blooming.

Thus, except for sporadic greening, these prairies were mostly in a condition of drought dormancy, with grass leaves rolled or folded. The short grasses, exclusive of the few flower stalks, were only 2–3 inches tall. In addition to light grazing by cattle (except at Phillipsburg), the leaf tips of the short grasses were often removed by grasshoppers, which occurred plentifully throughout the entire growing season, frequently at the rate of 6–15 per square foot. Little bluestem, wire grasses (*Aristida* spp.), and other mid grasses having

been killed by drought, the very open growth of short grasses alone partially covered the soil, except at Phillipsburg where short grasses alternated with side-oats grama. The impress of drought prevailed at all times. In fact, the much depleted cover, rather than being restored, apparently suffered further deterioration.

Grasses and forbs at the several stations

A census was made at each station, where soil samples were taken, of all species occurring within a radius of 100 feet. The lists of grasses and sedges and of forbs at the Iowa stations are shown in table 1. There are fifteen species in the first group. The forbs varied in number from thirty-seven to forty-eight at the individual stations and totaled sixty-five species. There were nineteen species of grasses at the Lincoln group of stations. Number of species of forbs varied from nine to nineteen and totaled thirty-three (table 2). In the group of wheat-grass prairies, the grasses and sedges were reduced to nine species. Species of forbs varied from eight to fifteen, with a total of twenty-one (table 3; figs. 14–17).

The forbs occurring at the three most westerly stations were few in number. Many less xeric species, common before the drought, were not found. The most evenly (although sparingly) distributed at the three sampling stations were *Malvastrum coccineum* and *Sideranthus spinulosus*. *Kuhnia glutinosa*, *Psoralea tenuiflora*, *Liatris punctata*, and *Cirsium undulatum* were found less abundantly, each at least at two of the three stations. *Allionia linearis*, *Allium nuttallii*, *Anemone caroliniana*, *Lygodesmia juncea*, and *Opuntia humifusa* occurred in the sampling area of at least one station. To this list of eleven perennial species must be added a half dozen (mostly native) annuals, previously mentioned, and the dominants *Bulbilis dactyloides* and *Bouteloua gracilis;* also at one station, *B. curtipendula*. Thus both reduction in number of species and sparseness of individuals toward the "dust bowl" were emphasized. Here, indeed, the web of life is stretched thin to cover the barren ground.

Increase in number of grasses from fifteen to nineteen from Iowa to Nebraska resulted from the gain of six xeric species (*Agropyron smithii*, two species of *Bouteloua*, *Buchloe dactyloides*, *Festuca octoflora*, and *Sporobolus asper*), but also included the loss of two (*Elymus*

TABLE 1

BLUESTEM PRAIRIES IN IOWA, SHOWING SPECIES OF GRASSES, SEDGES, AND FORBS AT EACH SAMPLING STATION: 1, GUTHRIE CENTER; 2, ANITA; 3, CORNING; 4, OAKLAND; AND 5, GLENWOOD

SPECIES	STATIONS					SPECIES	STATIONS				
	1	2	3	4	5		1	2	3	4	5
Andropogon furcatus	√	√	√	√	√	Euphorbia corollata	√	√	√	√	√
A. scoparius	√	√	√	√	√	Fragaria virginiana	√	√	√	. .	√
Bouteloua curtipendula	√	√	√	√	√	Gentiana puberula	√	. .	√	√	√
Carex festucacea	√	√	. .	√	√	Helianthus rigidus	√	√	√	√	√
C. pennsylvanica	√	√	√	. .	√	Heuchera hispida	. .	. .	√	. .	. .
Elymus canadensis	√	√	√	√	√	Hieracium longipilum	√	. .	√	. .	√
Eragrostis spectabilis	√	. .	. .	√	√	Houstonia angustifolia	. .	. .	√	. .	. .
Koeleria cristata	√	√	√	√	√	Kuhnia glutinosa	√	. .	√	√	√
Muhlenbergia cuspidata	. .	. .	√	√	. .	Lepachys columnaris	√	√	√	√	. .
Panicum scribnerianum	√	√	√	√	√	Lespedeza capitata	√	. .	. .	√	. .
P. wilcoxianum	√	√	√	√	√	Liatris pycnostachya	√	√	. .	. .	. .
Poa pratensis	√	√	√	√	√	L. scariosa	√	. .	. .	√	. .
Sorghastrum nutans	√	√	√	√	√	L. squarrosa	√	√	√	. .	. .
Sporobolus heterolepis	√	√	√	. .	. .	Linum sulcatum	√	. .	√	√	√
Stipa spartea	√	√	√	√	√	Lithospermum linearifolium	√	√	. .	√	. .
Acerates spp.	√	√	√	√	√	Lygodesmia juncea	√	. .	. .	√	√
Achillea occidentalis	√	. .	√	√	√	Meibomia canadensis	. .	√	. .	. .	. .
Amorpha canescens	√	√	√	√	√	M. illinoensis	√	√	. .	√	√
Anemone cylindrica	√	. .	√	√	. .	Mesadenia tuberosa	. .	. .	√	. .	. .
Antennaria campestris	√	√	√	√	√	Onosmodium occidentale	. .	. .	. .	√	. .
Artemisia gnaphalodes	. .	√	. .	. .	. .	Oxalis stricta	√	. .	. .	. .	√
Asclepias tuberosa	. .	√	√	. .	√	Pedicularis canadensis	. .	√	√	. .	. .
A. verticillata	√	. .	. .	√	√	Petalostemon candidus	√	√	√	√	√
Aster azureus	. .	. .	. .	. .	√	P. purpureus	√	. .	. .	√	√
A. laevis	√	. .	√	√	√	Phlox pilosa	. .	√	√	. .	√
A. multiflorus	√	√	√	√	√	Physalis lanceolata	√	√	√	√	√
A. oblongifolius	. .	. .	. .	. .	√	Psoralea argophylla	. .	√	√	. .	. .
A. sagittifolius	√	. .	√	√	√	P. esculenta	√	. .	. .	√	. .
A. sericeus	√	√	√	√	√	Rosa arkansana	√	√	√	√	√
Astragalus canadensis	. .	√	. .	. .	. .	Senecio plattensis	√	√	√	√	√
Baptisia leucophaea	. .	√	√	. .	. .	Silphium integrifolium	. .	. .	√	. .	. .
Ceanothus pubescens	√	√	√	√	√	S. laciniatum	√	. .	√	√	. .
Chamaecrista fasciculata	√	√	. .	. .	. .	Sisyrinchium angustifolium	√	. .	√	. .	√
Comandra umbellata	√	√	√	. .	√	Solidago glaberrima	. .	√	√	√	. .
Coreopsis palmata	√	√	. .	√	√	S. rigida	√	√	√	√	. .
Drymocallis agrimonioides	√	√	. .	√	√	S. rigidiuscula	√	√	. .	. .	√
Echinacea pallida	√	√	√	√	√	Vernonia baldwini	√	√	√	√	√
Equisetum laevigatum	√	√	. .	√	. .	Viola papilionacea	√	. .	√	. .	. .
Erigeron ramosus	√	√	√	√	√	V. pedatifida	√	√	√	√	. .
Eryngium yuccifolium	√	√	√	. .	. .	Zizia aurea	. .	√	√	. .	. .

canadensis, because of drought, and *Muhlenbergia cuspidata*, a xeric species not thickly distributed in the prairies). Reduction at the third group of stations to nine included the loss of six species by drought (*Andropogon scoparius*, *Carex festucacea*, *Panicum scribneri-*

TABLE 2

BLUESTEM PRAIRIES IN NEBRASKA AND KANSAS, SHOWING SPECIES OF PLANTS OCCURRING AT EACH SAMPLING STATION: 1, LINCOLN; 2, NELSON; 3, HEBRON; AND 4, BELLEVILLE

SPECIES	STATIONS				SPECIES	STATIONS			
	1	2	3	4		1	2	3	4
Agropyron smithii			√		Cirsium undulatum	√			
Andropogon furcatus	√	√	√	√	Drymocallis agrimonioides	√			
A. scoparius	√	√	√	√	Echinacea pallida	√			
Bouteloua curtipendula	√	√	√	√	Erigeron ramosus	√			
B. gracilis		√	√	√	Hedeoma hispida			√	
B. hirsuta		√	√	√	Helianthus rigidus	√			
Buchloe dactyloides		√	√	√	Kuhnia glutinosa	√	√	√	√
Carex festucacea		√		√	Lepachys columnaris			√	
C. pennsylvanica	√	√	√	√	Liatris punctata	√	√	√	√
Eragrostis spectabilis			√	√	Linum sulcatum		√		
Festuca octoflora		√	√		Lithospermum linearifolium	√			
Koeleria cristata	√	√	√	√	Meriolix serrulata	√	√		√
Panicum scribnerianum	√		√		Morongia uncinata				√
P. wilcoxianum	√				Opuntia humifusa			√	√
Poa pratensis	√				Oxalis violacea		√	√	
Sorghastrum nutans	√				Oxytropis lambertii		√		
Sporobolus asper	√		√	√	Parosela enneandra				√
S. heterolepis	√				Petalostemon candidus	√	√		√
Stipa spartea	√				P. purpureus		√		√
					Physalis lanceolata	√			
Acerates angustifolia	√				Rhus toxicodendron				√
Allionia linearis				√	Rosa arkansana	√			√
Amorpha canescens	√	√	√	√	Senecio plattensis	√			
Anemone caroliniana		√			Solidago glaberrima	√	√		
Artemisia gnaphalodes		√	√	√	S. rigida	√			
Aster multiflorus	√	√	√	√	Vernonia baldwini		√		√
Baptisia leucophaea	√								

anum, *P. wilcoxianum*, *Poa pratensis*, and *Sorghastrum nutans*). *Bouteloua hirsuta*, *Eragrostis spectabilis*, and *Sporobolus heterolepis* formerly occurred, if at all, only sparingly. No new species were gained. A final reduction to only four grasses at the western Kansas stations resulted from death of all the mid grasses which formerly occurred there except *Bouteloua curtipendula*.

Among the grasses, *B. curtipendula* alone was found at all four groups of stations, thus illustrating its wide adaptability and drought-resistant qualities. Conversely, *Stipa spartea* occurred only at the first two, but the deeply rooted *Andropogon furcatus* and the rapidly reproducing *Koeleria cristata* at all but the last. *Buchloe dactyloides* and *Bouteloua gracilis*, most xeric of all, occurred at all three western

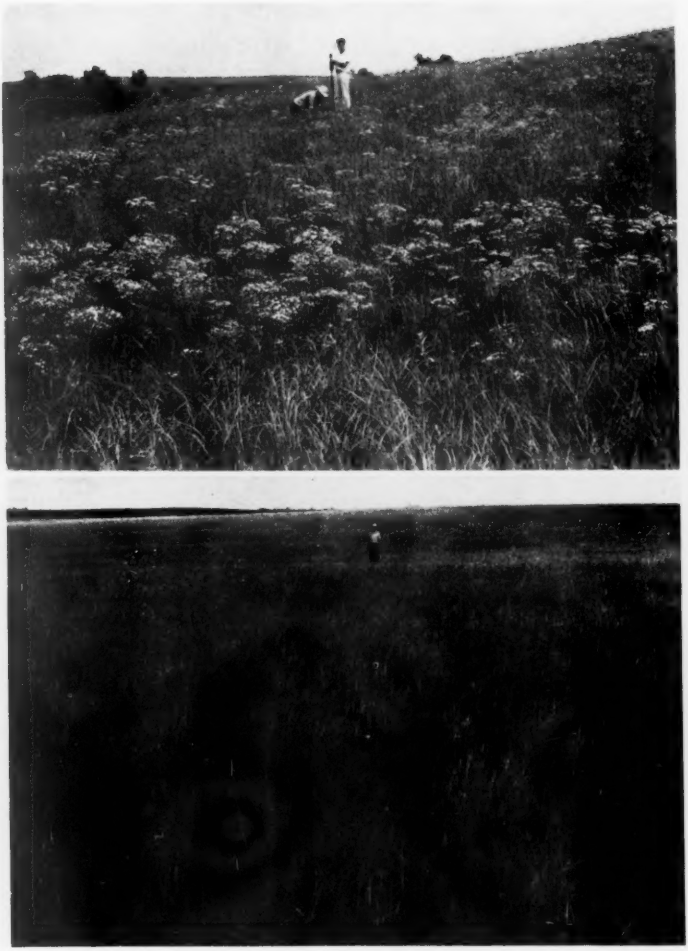
TABLE 3

WHEAT-GRASS PRAIRIES IN NEBRASKA AND KANSAS, SHOWING SPECIES OF PLANTS OCCURRING AT EACH SAMPLING STATION: 1, CLAY CENTER; 2, CARLETON; AND 3, MONTROSE

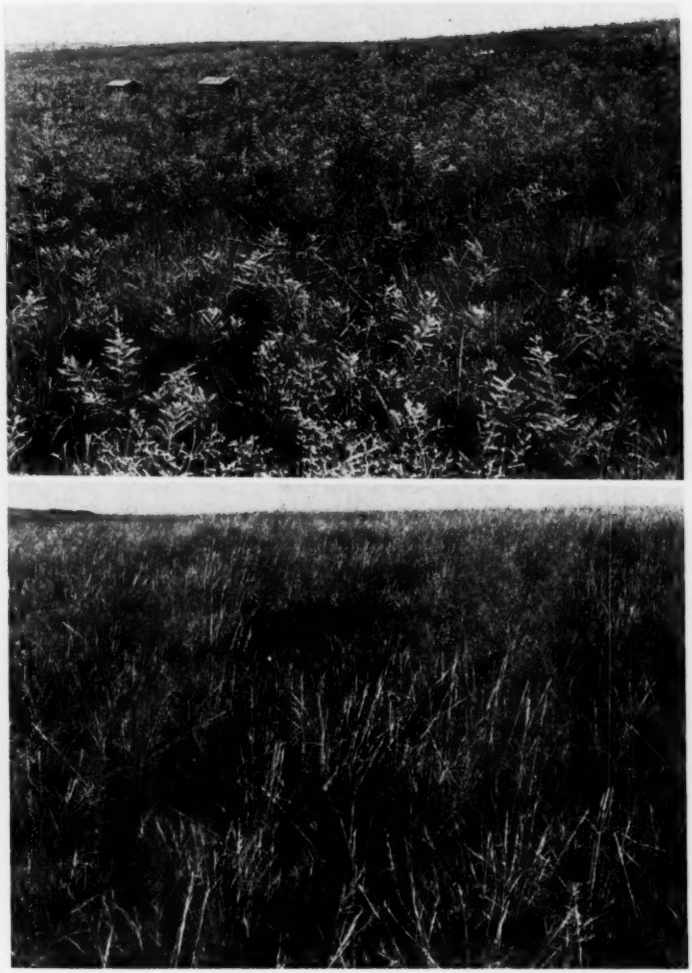
SPECIES	STATIONS			SPECIES	STATIONS		
	1	2	3		1	2	3
Agropyron smithii	√	√	√	Cirsium undulatum	√	...	...
Andropogon furcatus	...	√	√	Kuhnia glutinosa	√	√	√
Bouteloua curtipendula	√	√	√	Liatris punctata	√	√	...
B. gracilis	√	√	√	Lygodesmia juncea	√	...	√
Buchloe dactyloides	...	√	√	Malvastrum coccineum	√	...	...
Carex pennsylvanica	√	√	√	Meriolix serrulata	√	√	...
Festuca octoflora	...	√	...	Oxalis violacea	√	√	√
Koeleria cristata	√	√	...	Physalis lanceolata	...	...	√
Sporobolus asper	...	√	...	Rosa arkansana	...	√	√
				Salvia pitcheri	...	...	√
Acerates angustifolia	√	√	√	Senecio plattensis	√	...	...
Allionia linearis	...	...	√	Sideranthus spinulosus	...	...	√
Amorpha canescens	√	√	√	Solidago glaberrima	√	...	...
Artemisia gnaphalodes	√	...	...	S. mollis	√	...	√
Asclepias verticillata	√	...	...	Vernonia baldwini	...	...	√
Aster multiflorus	√	√	√				

groups of stations; in fact, they are rapidly spreading to replace mid grasses.

Reduction of forbs from sixty-five in Iowa to thirty-three in the Lincoln group occurred notwithstanding the gain of a total of nine species, including the xeric *Allionia linearis*, *Cirsium undulatum*, *Opuntia humifusa*, and *Oxytropis lambertii*. The further reduction to twenty-one species was accompanied by the addition of three western ones—*Malvastrum coccineum*, *Sideranthus spinulosus*, and *Solidago mollis*. The final group of only eleven perennial forbs includes two xeric species (*Psoralea tenuiflora* and *Allium nuttallii*) not found in the preceding sampling areas.



FIGS. 14, 15.—Fig. 14 (above), prairie at Anita, Iowa, August 15, 1939, showing abundance of forbs, most conspicuous of which is *Euphorbia corollata*. Fig. 15 (below), prairie near Lincoln, Nebraska, August 1, 1939, showing almost entire absence of forbs, typical of conditions at all but the Iowa stations.



FIGS. 16, 17.—Fig. 16 (above), portion of Belmont prairie at Lincoln, July 6, 1932, showing abundance of *Amorpha canescens* and other forbs in cover of *Andropogon scoparius*. Fig. 17 (below), same area on July 6, 1939. Note great decrease in forbs and replacement of little bluestem by *Stipa spartea* and other more xeric grasses.

Six species of the ground layer or understory occurred only at the most mesic group of stations, although they were formerly plentiful in the Lincoln group also, some species being common even farther westward. These are *Antennaria campestris*, *Sisyrinchium angustifolium*, *Viola pedatifida*, *V. papilionacea*, *Fragaria virginiana*, and the rosettes of *Hieracium longipilum*. Clearly this depletion of the ground layer was an effect of drought. Three other understory species (*Carex pennsylvanica*, *Oxalis violacea*, and *Anemone caroliniana*) not only persisted but increased in abundance, probably because of underground storage organs (4).

A number of very deeply rooted species occurred at all but the most xeric group of stations. These were *Amorpha canescens*, *Rosa arkansana*, *Vernonia baldwini*, *Solidago glaberrima*, and *Aster multiflorus*. *Senecio plattensis* and *Artemisia gnaphalodes* accompanied them. *Kuhnia glutinosa*, which is very drought resistant, probably because of its excellent, deeply seated taproot, was the only species found at all four station-groups. In fact, it occurred at each of eleven stations, although never abundantly. Other species found at the three more xeric groups of stations, and which are notably drought resistant, are *Liatris punctata*, *Cirsium undulatum*, *Allionia linearis*, and (absent in the Lincoln group) *Lygodesmia juncea*. The equally xeric *Malvastrum coccineum* and *Sideranthus spinulosus*, found only at the two driest station-groups, are not naturally distributed so far eastward as the preceding.

It is of interest and importance that of the eleven Leguminosae in Iowa, the number was reduced first to seven, and then to one at each of the more westerly station-groups. The much more regular occurrence of many species at all the stations in the Iowa group, unaffected by drought, is also significant. Of the total of fifteen grasses or grasslike species, thirteen were found at each station, but only ten to thirteen of a total of nineteen at each station of the Lincoln group.

These data, presented from the relatively small but numerous sampling areas, are in general substantiated by much more extensive surveys, including entire prairies, which are now under way. They indicate clearly some of the drastic changes in plant populations resulting from the years of drought. A loss of 50–66 per cent

of forbs was found in 1938; during 1939 they have been further depleted both in numbers and species.

The value of this study of environmental relations, density of plant cover, distribution of forbs, etc.—although done intensively only in this central area of the mid-continental grasslands—is enhanced by the fact that it represents generally the great changes that have been wrought in the grasslands from east to west by the several years of drought.

Summary

1. Four groups of prairies of known previous history, extending from southwestern Iowa to western Kansas, were studied in 1939.

2. The Iowa group (precipitation about 32 inches) had been scarcely affected by the great drought. At one Nebraska-Kansas group, bluestem grasses (*Andropogon* spp.) still prevailed; at the other they had died and been replaced by *Agropyron smithii*. *Bouteloua gracilis* and *Buchloe dactyloides* were the chief grasses of the western Kansas prairies (precipitation about 22 inches).

3. The twelve prairies in the four groups all occurred on deep, fertile, silt-loam soils; water content was determined monthly to a depth of 6 feet.

4. The bluestem prairies of Iowa, where rainfall was plentiful, had a continuous supply of available water at all depths; those of Nebraska and Kansas had only a low supply after early spring and were repeatedly threatened with drought. In the wheat-grass group, available deep-soil moisture was the exception, and repeated exhaustion of surface soil moisture occurred. Water was available only in the surface soil at the short-grass stations and at only two or three periods.

5. Slightly higher temperatures, 50–100 per cent more evaporation, and two to three times as much wind movement occurred in western Kansas as in eastern Nebraska.

6. No previous deterioration of vegetation had occurred in Iowa. The ground layer had been destroyed and the basal area was only one-half to two-thirds normal in the drought-depleted bluestem prairies westward. Drought and dust had destroyed most of the former plant cover of the third group of prairies, which were now

dominated by an open growth of western wheat grass which permitted a continuous pattern of bare soil. In western Kansas the 85 per cent basal cover of short grasses had been reduced by continued drought, burial by dust, and injury by grasshoppers to 10–15 per cent. The remaining soil was bare.

7. Grasses grew normally in Iowa, reaching a foliage level of 18 inches; they dried after midsummer and failed to flower in eastern Nebraska. Wheat grass dried very early and burned readily in July. The short grasses in west-central Kansas were dormant during most of the summer but grew to a height of 3 inches when revived by late summer showers.

8. In Iowa, fifteen species of grasses and sedges occurred at the sampling stations, but nineteen at the western, bluestem station-group. There were but nine at the wheat-grass stations and only four in the short-grass areas.

9. Native forbs similarly decreased from sixty-five to thirty-three species, then to twenty-one, and finally to eleven.

10. Conditions of the central area of the mid-continental grassland reveal in general changes that have been wrought elsewhere from east to west by continued drought.

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SIZE OF NUCLEI IN THE SHOOT OF RICINUS COMMUNIS

FLORA MURRAY SCOTT

(WITH SEVEN FIGURES)

Introduction

While working on the differentiation of the spiral vessels in *Ricinus* (2), the extremely large size of the nuclei within the coenocytes was noticed. It was decided to examine further the question of nuclear size in the shoot as a whole. MONSCHAU (1) has shown that, in the case of certain typical monocotyledons and dicotyledons, the nucleus of parenchyma cells such as pith and cortex measures approximately eight times the volume of the meristem nucleus. Statistical evidence given in this paper indicates that volume increase is effected by doubling and redoubling of nuclear material.

In *Ricinus* the volume of the meristem nuclei is approximately 38 cu. μ, that of the majority of nuclei of pith and cortex 332 cu. μ, an eightfold increase. In marked contrast to this, the largest nuclei of the developing spiral vessels, and also of the secondary vessel segments, may measure as much as 59,000 cu. μ, or 1550 times the original nuclear volume.

In the present paper a survey is made of the nuclear volume in the shoot as a whole, with particular stress on the "giant" nuclei of spiral elements and of the pitted vessel segments of the primary and secondary tissues, respectively, in axis and in petioles. Certain preliminary evidence in regard to the nature of the components responsible for volume increase—chromatin, nucleolus, karyolymph, and water—is also presented.

Material and methods

Shoot tips and secondary tissues of actively growing plants of *Ricinus communis* were fixed in Navashin's solution, imbedded, sectioned, and stained either with haematoxylin or with safranin and fast green. Sections were necessarily cut 10–60 μ thick, depending on the region of the axis and the vessel diameter. Measurements

were made both of fresh material and of fixed preparations. The nuclei are spherical or ellipsoidal, and volumes are calculated from the formulas $\frac{4}{3}\pi r^3$ and $\frac{4}{3}\pi a^2 b$.

In determining nuclear components, mitotic figures were examined in coenocytes and in meristematic tissues. The varying volumes of the nucleoli were noted in relation to the size of the containing nuclei. The relative amount of water present in larger and smaller nuclei was estimated by the volume contraction induced by irrigation with absolute alcohol, followed or not by xylene.

Observations

Volume of nuclei of spiral elements and diameters of spiral vessels

The spiral elements, like other primary vascular cells, differentiate from procambial tissue but pass through a characteristic coenocytic phase, the details of which have been previously described (3).

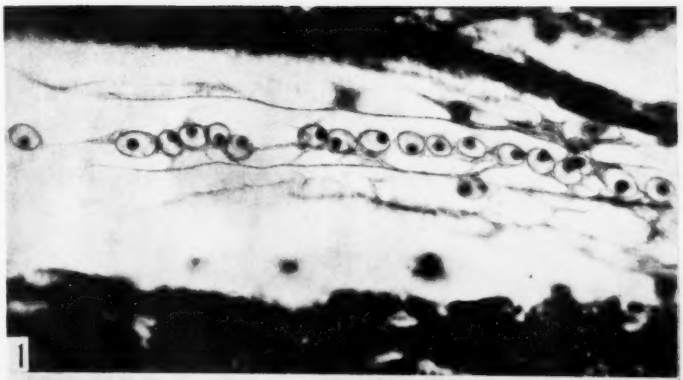
TABLE 1

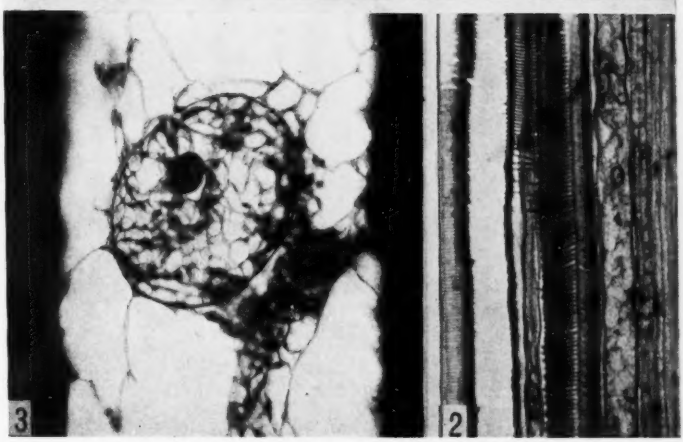
NUCLEAR VOLUMES (IN ROUND FIGURES) OF SPIRAL VESSELS IN 1000 CU. μ GROUPS AND THEIR FREQUENCIES

Volume.....	0–999	1000	2000	3000	4000	5000	6000	7000	8000	9000
Frequency....	570	313	125	71	31	21	17	17	8	8
Volume.....	10,000	11,000	12,000	13,000	14,000	16,000	17,000	18,000	20,000	30,000
Frequency....	8	5	5	1	4	1	4	1	4	1
Volume.....	40,000	50,000								
Frequency....	1	1								

Coenocytes may show from two to seventeen or more nuclei (fig. 1). The nuclei persist after the spiral thickening is laid down (fig. 2). Nuclear measurements are made in the developing coenocytes in all stages of development.

Nuclear volumes increase in passing from younger to older internodes. In the shoot as a whole the volume measurements form a continuous series from 38 to 59,000 cu. μ. Certain volumes, however, occur more frequently than others. The range of nuclear volume in the petiole is similar to that in the shoot. When volumes are arranged in size groups of 1000 cu. μ, it is seen that the greatest number of nuclei do not exceed 2000 cu. μ in volume (table 1). When the volumes are plotted in detail, however, up to 4000 μ a many-peaked graph is the result (table 2 and fig. 4). The maximal frequencies in





FIGS. 1–3.*—Fig. 1, longisection of stem apex showing multinucleate coenocyte of differentiating spiral vessel. Fig. 2, older internode showing coenocyte adjacent to lignified vessels, protoplast with nuclei present in one of the latter. Fig. 3, same showing reticulum of nucleus, nucleolus, nuclear sap, etc., in "giant" nucleus, adjacent to fully lignified vessel.

* Since only one optical level in a section can be accurately focused at any one time, details of protoplasts of photomicrographs are outlined for the sake of greater clearness.

the first section of the graph occur at 332, 585, 755, 1146, and 1360 cu. μ. Thereafter with increasing volume the lesser maxima appear

TABLE 2

NUCLEAR VOLUMES IN SPIRAL VESSELS IN INTERNODES AND IN PETIOLES (IN CU. μ) AND THEIR FREQUENCIES (ABBREVIATED); 1212 MEASUREMENTS

VOLUMES 38–4000

Volume.....	332	385	755	1073	1146	1360	1640	2087	2743	3159	3953
Frequency.....	94	36	78	34	55	70	33	17	18	14	9

VOLUMES 5000–10,000 (in round figures)

Volume.....	±5500	±6500	±7500	±8500	±9500	±10,500
Frequency.....	21	17	17	8	8	8

VOLUMES 11,000–20,000

Volume.....	±11,500	±12,500	±13,500	±14,500	±16,500	±17,500	18,500	±20,000
Frequency.....	5	5	1	3	2	3	1	4

VOLUMES 30,000–60,000

Volume.....	32,013	48,398	57,226	59,550
Frequency.....	1	1	1	1

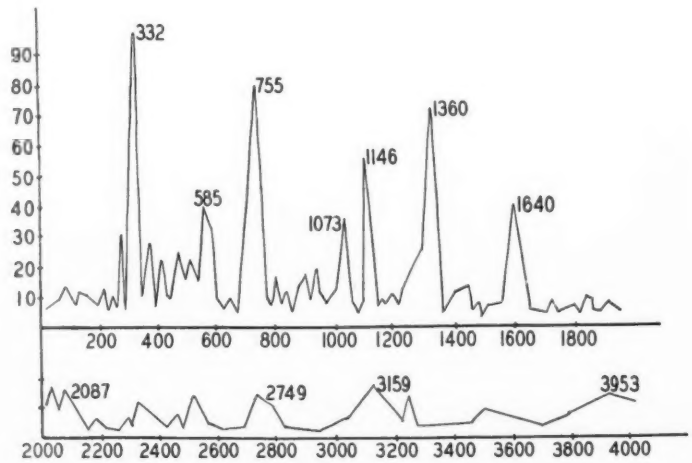


FIG. 4.—Volumes of spiral vessel nuclei of internodes and petioles (32–4000 cu. μ only) and their frequencies (cf. table 2).

with less regularity, and, if continued above 5000 cu. μ, the curve would flatten to reach the maximum volume of 59,550, a 1550-fold increase. The decreasing numbers in this region are due in part to greater technical difficulties in dealing with thicker sections.

The nuclei of the differentiating vessels are spherical or ellipsoidal. The diameters or minor axes of the nuclei of maximum frequency are given in table 3, and are followed by those of the larger nuclei, which are fewer in number.

The nuclear diameters of all nuclei thus range from 6 to 35, and the diameters of the volumes up to 3000 are (in round numbers): 6, 7, 9, 10, 13, 17.

TABLE 3

Volume	Diameter (μ)	Volume	Diameter (μ)
Nuclei of maximum frequency			
332	8.7	2749	17.4
585	10.4	3159	13.0
755	6.9	3261	8.7
1146	13.0	3511	13.0
1360	8.7	3953	10.4
1640	10.4	4210	17.4
2048	13.0	4937	13.0
2087	8.7	5405	11.3
2530	10.4		
Larger nuclei			
6237	13.0	15,349	15.6
7900	16.5	17,598	21.7
9873	17.4	33,544	17.4
12,936	27.8	59,550	34.8

When nuclei are measured *in situ* it is seen that the diameter of the largest measures slightly more than half that of the containing vessel or coenocyte. In the largest vessels, 70–180 μ diameter, this ratio is not sustained. The increase in volume of the largest ellipsoidal nuclei is due to increase along the major axis.

Since nuclei are spherical or ellipsoidal, it is apparent that their volumes in the same coenocyte, and in different coenocytes of the same diameter, may vary widely. The arrangement of larger and smaller nuclei within a single coenocyte appears to be haphazard (table 5). There is no gradation in size from one end of the coenocyte to the other.

NUCLEI OF SECONDARY VESSEL SEGMENTS

The vessel segments of the secondary xylem measure 250–400 μ in length and 60–110 μ in width. The perforated end walls are generally

TABLE 4

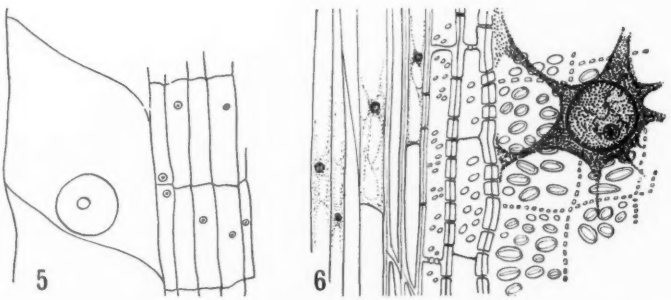
DIAMETERS OF NUCLEI IN RELATION TO VESSELS; 217 MEASUREMENTS

Vessel diameter (μ)	15	17	21	26	30	34	39	44	52	60
Range of nuclear diameters	6–10	6–11	8–12	8–13	8–17	8–18	9–20	11–26	13–26	17–30

TABLE 5

VARIATIONS OF NUCLEAR VOLUMES IN SAME COENOCYTES; NUCLEI LIE ADJACENT IN PLASMOLYZED PROTOPLASTS

1	2	3	4	5
585	755	755	383	816
3050	2530	483	1360	755
1146	2087	332	483	3261
483	732	585	560	291
2749	332	613	755	1073
			430	1073
			358	



FIGS. 5, 6.—Fig. 5, longisection of secondary xylem (camera lucida) showing contrast between nuclear size in cambium and in differentiating vessel segment. Fig. 6, same showing border-pitted wall in lignified vessel segment; protoplast still present.

steeply oblique, and the vertical walls are heavily pitted with bordered, or occasionally simple, pits (figs. 5, 6). In a single longitudinal radial section it is possible to observe both cambial and vessel nu-

clei. The latter have a range in volume equal to that found in the spiral vessels. Volumes of 7629, 16,428, 22,075, 25,196, 55,351 are among those recorded. The former, the cambial nuclei, retain the initial volume of the meristem nuclei, 38 cu. μ.

Nuclei of parenchyma

In the first 20 cm., approximately twelve to fifteen internodes, the nuclei of the pith are readily observed. Below this point starch-containing plastids form an envelope around the nucleus and obscure accurate definition. In the younger internodes the nuclei range from

TABLE 6

Volumes of pith nuclei and their frequencies

0–99	100	200	300	400	500	600–999	1000	2000	3000	4000
38–7	100–3	215–1	312–1	407–1	560–1	656–1	1146–25	2058–2	3084–2	4180–3
58–1	112–29	230–1	332–53	430–14	585–23	732–12	1314–1	2200–2		
73–35	124–4	237–1	358–1	483–15		755–1		2394–1		
97–4	133–1	247–2	383–3			772–2		2464–4		
	144–4	261–5				902–2		2620–1		
	164–38	275–1				918–4		2749–2		
		299–1						2848–1		

38 to 332 cu. μ in volume. In the older internodes the volume may reach 4000 cu. μ (table 6).

Plotting of volume-frequency from a more limited number of measurements (300) results, as in the case of the vessel nuclei, in a many-peaked graph, with maxima at 73, 164, 332, and lesser maxima at 112, 585, and 1146 (fig. 7). If the volume of the meristem nucleus, 38 cu. μ, is regarded as unit volume = x, then the first three maxima appear as approximate multiples of this figure, 2x, 4x, 8x, the actual multiples being 76, 153, 304. This is in agreement with the findings of Monschau (2). The lesser maxima, forming what is essentially a continuous series, represent approximate multiples—3x, 15x, 30x. The largest nuclei occur in the older internodes and range from 500 to 4000 cu. μ, but thus do not approach the volume of the largest vessel nuclei. The nuclei of cortex, rays, and other tissues lie within the same general range as the nuclei of the pith.

NUCLEOLUS

The nucleolus varies relatively little in size and does not keep pace with the rapidly enlarging nucleus. While the nucleus increases from 38 to 3000 cu. μ, the nucleolus does not change appreciably from its initial volume, 19 cu. μ. Thereafter as the nucleus reaches 9000, 17,000, and 33,000 cu. μ, the nucleolus measures 71, 330, and 943. In the largest nuclei, those over 10,000 cu. μ, there is no constant relation between nuclear and nucleolar size. This ratio may range erratically from 35:1 to 298:1 (table 7).

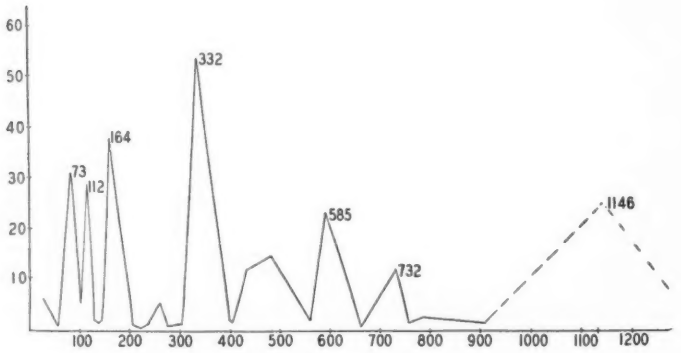


FIG. 7.—Volumes of nuclei of pith and their frequencies (30–1200 μ only)

CYTOLOGY OF NUCLEI

An important question in regard to the giant nuclei is, To what material is due the enormous increase in bulk—chromatin, nucleolus, karyolymph, or water? (fig. 3).

1. CHROMATIN.—In meristematic nuclei the number of chromosomes observed is nineteen. In developing coenocytes a certain number of mitotic divisions has been observed in metaphase and anaphase condition. The number of chromosomes has not increased, therefore the increase in volume is not attributable to a polyploid condition. The size of the individual chromosomes appears to remain constant, and the bulk of chromatin material as a whole when measured in anaphase is not significantly greater. Increased volume therefore does not depend on increased chromatin.

2. NUCLEOLUS.—The size of the nucleolus has already been discussed and its variability in larger nuclei noted. The nucleolus does not contribute significantly to increased nuclear volume.

3. KARYOLYMPH, WATER.—The addition of absolute alcohol to fresh material causes marked contraction in nuclear size. The addi-

TABLE 7

RATIO OF VOLUME OF NUCLEUS TO VOLUME OF NUCLEOLUS

VOLUME OF NUCLEUS	VOLUME OF NUCLEOLUS	RATIO
332	19	16:1
585	19	28:1
755	19	39:1
1360	19	71:1
1360	38	35:1
2087	19	109:1
2087	38	54:1
2530	19	133:1
2530	38	66:1
3159	38	83:1
4937	38	127:1
9126	71	128:1
10,569	196	54:1
10,731	71	151:1
11,051	37	298:1
11,499	58	198:1
11,895	163	72:1
12,617	112	112:1
14,157	96	147:1
14,637	259	56:1
17,599	330	53:1
34,544	943	35:1

tion of xylene does not induce further shrinkage. It is assumed that water is the only component removed from the nucleus in this dehydration process. Whether the contraction indicates the total volume of free water remains an open question. The nucleolus does not change appreciably in volume in the nuclei observed. Shrinkage in volume on dehydration varies in meristematic and in differentiated nuclei, as shown in tables 8 and 9.

In meristem cells between which intercellular spaces have not yet developed, the contraction is approximately 42 per cent. In the nuclei of the meristematic dividing region, intercellular spaces ap-

pear and vacuolation of cells has begun. Shrinkage ranges from 27 to 81 per cent. In the pith and spiral vessel nuclei the same variability in contraction is evident. The spiral vessel nuclei show a fairly consistent increase in water loss with increasing size.

TABLE 8

CONTRACTION OF NUCLEI ON ADDITION OF ABSOLUTE ALCOHOL

MATERIAL	ORIGINAL VOLUME* (CU. μ)	VOLUME AFTER CONTRACTION (CU. μ)	PERCENTAGE	
			CONTRACTION	RESIDUAL VOLUME (CU. μ)
Stem apex: meristematic dividing cells; no vacuoles present	136	81	40	60
	112	65	41	59
	112	65	41	59
	94	52	43	57
Stem apex: vacuolating dividing cells; intercellular spaces developing	112	65	41	69
	112	65	41	69
	112	65	41	69
	65	33	47	53
	65	33	47	53
Stem apex: same as preceding	372	270	27	33
	687	425	38	62
	410	171	58	42
	522	94	81	19
Pith of eleventh and thirteenth internodes	522	233	55	45
	1146	522	54	46
	1761	1146	54	46
	1879	1337	28	72
	2280	1761	22	78
	2555	1526	40	60
	5543	2140	61	39
	5543	1761	68	32

* Nucleolus included in the volumes.

These preliminary findings indicate that increase in volume is accounted for to a great extent by increased water content. The remaining increase in size over and above that accounted for by water must therefore be attributed to karyolymph. Compare, for example, the nuclei of volumes 10,344 and 51,128 with their volumes after dehydration, 2056 and 9202. The smallest meristematic nuclei measure (minus the nucleolus) 34 cu. μ. If the water content of such a nucleus is assumed to be 40 per cent, then the volume of karyolymph

and chromatin measures 22 cu. μ. In the nuclei under discussion a 200-fold and a 400-fold increase in the karyolymph volume is therefore apparent.

Time of mitotic division

The time of mitotic division is undetermined. Since young coenocytes occur with as many as sixteen to twenty-two small nuclei of

TABLE 9

Contraction of nuclei continued

Material	Original volume (cu. μ)	Volume after contraction (cu. μ)	Percentage	
			Contraction	Residual volume (cu. μ)
Spiral vessel nuclei	253	119	52	48
	253	119	52	48
	570	344	39	61
	738	266	63	37
	738	103	86	14
	1148	266	76	24
	1148	266	76	24
	1226	344	71	29
	1226	344	71	29
	1315	266	79	21
	2780	738	73	27
	6025	3861	49	51
	7642	1894	68	32
	10,344	2056	80	20
	12,302	4460	64	36
	27,254	4961	81	18
	51,128	9202	82	28

Volume of nucleolus subtracted in the volumes.

identical size adjacent in the same protoplasmic strand, it is presumed that nuclear division takes place repeatedly in the earliest stages of differentiation (fig. 1); but mitotic division is also occasionally observed in coenocytes of larger diameter.

The preliminary data on nuclear contraction already cited thus indicate that meristematic activity may be definitely linked with limited water content.

Summary

1. During differentiation of the primary spiral vessels of *Ricinus communis* the nuclei of the coenocytes become extremely large. The volume of typical meristem nuclei is 38 cu. μ, that of spiral vessel

nuclei may range up to 59,000 cu. μ, a 1550-fold increase in volume. The nuclei of maximal frequency do not fall into the size groups of MONSCHAU, K1:K2:K3:K4 of volume ratios 1:2:4:8, in which each class is a multiple of the preceding. Nuclear and vessel diameter are interdependent except in the largest vessels. The nuclei attain their maximum size at the beginning of the process of spiral thickening, remaining intact and presumably functional for some time after lignification is complete.

2. In the younger internodes of the pith the nuclei fall into a series approximating MONSCHAU's size groups, with a maximum frequency at 332 cu. μ, an eightfold volume increase. In older internodes they range in size up to 4000 cu. μ.

3. In regard to the material responsible for nuclear size, it is seen that: (*a*) In mitotic divisions the nucleus remains diploid and the chromosomes are not significantly greater than those of the meristem. (*b*) The nucleolus does not keep pace with the increasing size of the nuclei, and in the largest nuclei the nucleolar-nuclear ratio is highly variable. (*c*) Karyolymph increases 200- to 400-fold in volume. Water removable by the addition of absolute alcohol may constitute as much as 80 per cent of nuclear volume. Increase in volume is therefore attributable to these two factors.

4. Meristematic activity and nuclear water content appear to be interdependent.

5. It seems improbable that the vessel nuclei of *Ricinus* are unique in their nucleo-cytoplasmic ratio. Re-examination of thicker sections of fresh and fixed material of other large-vesseled plants may reveal conditions similar to that in *Ricinus*.

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GROWTH RESPONSES OF BILOXI SOYBEANS TO VARIATION IN RELATIVE CONCENTRATIONS OF PHOSPHATE AND NITRATE IN THE NUTRIENT SOLUTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 510

CHARLES L. HAMNER

(WITH FOUR FIGURES)

Introduction

Experimental results from many nutrient experiments have shown that, in general, if an element is lacking or low in concentration in the nutrient solution or soil solution, it will be lacking or present only at very low concentrations in the plant. Also, an essential element present in abundance in the nutrient solution will probably be present in abundance in the plant, although its concentration may vary with variation in environmental factors. A factor which may affect the accumulation of one element in the plant is the presence or absence of another essential element in the nutrient solution (2). The deficiency of an element or its abundance in the nutrient solution may affect the rate and amount of absorption of other elements, and thus affect growth responses.

It is probable that for every plant there is a specific nutrient requirement for maximum vegetative expression, flowering, fruit production, etc., and it is probable that this requirement changes as the conditions within the plant or external environments change. Just what constitutes optimum requirements will depend upon the viewpoint of the investigator, but many workers have utilized vegetative growth responses as a measure of the suitability of any particular nutrient solution. Two methods of measuring the growth responses of plants are possible, and may be classified as qualitative and quantitative. Quantitative measurements are concerned with the total accumulation of dry matter by the plant; qualitative measurements are concerned with the character or types of material composing the plant—proteins, fats, vitamins, etc. After a definite corre-

lation between quantitative response and nutrient requirements has been established, then an investigation of the qualitative responses may lead to an understanding or interpretation of the quantitative responses.

The object of the present study was a determination of the relationships existing between growth responses and the concentration of various ions in sand and solution culture. Since it is impossible to measure and record all the growth responses of any particular group of plants, and since the summation of the qualitative responses represent the quantitative responses, the criteria of response used here have been the accumulation of dry matter by the plant and its parts and the general appearance of the plant.

Experimental methods

Biloxi soybeans were used as experimental material. Several preliminary experiments were carried out, both in sand and solution culture, but the detailed results presented have been taken only from sand culture experiments, since plants grew as well in sand culture as in comparable solution cultures and the former experiments were more readily carried out. In all experiments, seeds were selected for uniformity and planted at an even depth in well-washed white quartz sand. They were then placed in darkness or in the greenhouse and watered with tap water. After germination, uniform seedlings were selected and transplanted to quartz sand contained in glazed 8×4-inch crocks with a $\frac{3}{8}$-inch hole in the bottom, each crock containing about 1500 cc. of sand. They were maintained on a well lighted greenhouse bench. After transplantation, the plants were irrigated with the various nutrients. The preliminary experiments were carried out in the early summer when the length of a natural day was in excess of the critical photoperiod (**4**). The final experiments were carried out in the late summer and the plants received supplementary illumination of an intensity of about 20 foot-candles from sunset to 2 A.M., so that the plants remained vegetative throughout the experimental period. No attempt was made to control greenhouse temperatures, although excessively high temperatures were avoided by careful ventilation. Excessively low atmospheric humidity was prevented by frequent spraying of the walks

and walls with water during warm dry days. The plants were watered each day with sufficient nutrient solution so that an excess dripped out of the openings in the bottom of the crocks; twice a week the pots were thoroughly flushed with distilled water, immediately followed by application of fresh nutrient solution.

Harvesting

In preliminary experiments, observations were made on the effect of an increase in the concentration of a single one of the major essential elements of the nutrient solution by adding its sodium salt or its chloride, depending upon whether the element under consideration was an anion or a cation. The procedure was to use a three salt solution (**8**), which promotes fairly active vegetative growth, but to use this at about one-fifth the concentration ordinarily employed. Growth of plants in this solution was compared with the growth obtained by adding various single salts to it.

The various nutrient solutions used and the results obtained are shown in table 1. The experiment was continued for 16 days after transplanting.

The result of the preliminary experiment showed that with a concentration one-fifth that of the complete nutrient solution used, or even with one-tenth concentration of the complete solution, active vegetative growth could be obtained without any deficiency symptoms appearing. The complete solution diluted to one-fifth concentration, to which KCl had been added, showed the greatest growth next to the complete solution. The complete solution diluted to one-fifth, to which NaH_2PO_4 had been added, showed evidence of toxicity. The complete solution diluted to one-fifth, to which NaCl had been added, also showed poor growth (table 1). It was not possible to tell whether the decreased growth was caused by Na or by Cl or both. It was also not possible to decide whether the toxicity was caused by Na or by the HPO_4.

Because of the difficulty of interpreting the results of the preliminary experiments, another method was utilized in making up the nutrient solutions. By this procedure two series of nutrient solutions were obtained. In one series the cations (Ca, Mg, and K) would vary in relation to one another while the anions (S, N, and P)

remained constant throughout the series. In the other, the anions varied in relation to one another while the cations remained constant. Thus two nutrient triangles, similar in some ways to those presented by SHIVE (**8**), were utilized. The advantage of this system was that the concentration of only three ions varied from square to square in any given triangle (fig. 1), while in SHIVE's system there

TABLE 1

EFFECT OF ADDITION OF SINGLE SALTS TO STANDARD THREE SALT NUTRIENT SOLUTION ON GROWTH OF BILOXI SOYBEANS (PER 100 PLANTS)

COMPOSITION OF NUTRIENT SOLUTION	TOTAL DRY WEIGHT (GM.)	DRY WEIGHT OF TOPS	DRY WEIGHT OF ROOTS	TOP/ROOT RATIO
A. Complete solution ($MgSO_4$ 0.0045 mol; $Ca(NO_3)_2$ 0.0060 mol; KH_2PO_4 0.0045 mol)	101.97	80.57	21.4	3.76
B. One-fifth complete; same as A but at one-fifth concentration	77.6	47.7	30.0	1.59
C. Same as B with 0.0108 mol $CaCl_2$ added	66.66	47.57	19.09	2.49
D. Same as B with 0.0216 mol $NaNO_3$ added	64.69	48.53	16.16	3.00
E. Same as B with 0.0081 mol $MgCl_2$ added	65.55	44.72	20.83	2.14
F. Same as B with 0.0081 mol Na_2SO_4 added	72.06	47.06	25.00	1.88
G. Same as B with 0.0081 mol KCl added	82.88	56.76	26.17	2.16
H. Same as B with 0.0081 mol NaH_2PO_4 added	48.33	33.33	15.00	2.22
I. Same as B with 0.0216 mol NaCl added	53.99	36.28	17.71	2.05
J. Same as A but at one-tenth concentration	67.97	40.27	27.77	1.45

is variation in six ions, a variation in a cation always being accompanied by an equal variation in a corresponding anion. The accomplishment of this involved the use of two triangle systems.

In one triangle (fig. 1) a series of nutrient solutions in which the anions varied were made up using three stock solutions. Solution A consisted of 0.0045 mol KNO_3, 0.0045 mol $Mg(NO_3)_2$, and 0.0060 mol $Ca(NO_3)_2$. Solution B consisted of 0.0045 mol K_2SO_4, 0.0060 mol $CaSO_4$, and 0.0045 mol $MgSO_4$. Solution C consisted of 0.0060 mol $Ca(H_2PO_4)_2$, 0.0045 mol $MgHPO_4$, and 0.0045 mol KH_2PO_4. Plants

at one apex received solution A; at another apex, solution B; and at the third apex, solution C. The solutions in the body of the triangle were made by mixing various proportions of solutions A, B, and C, as indicated in figure 1. The concentrations of cations in the three

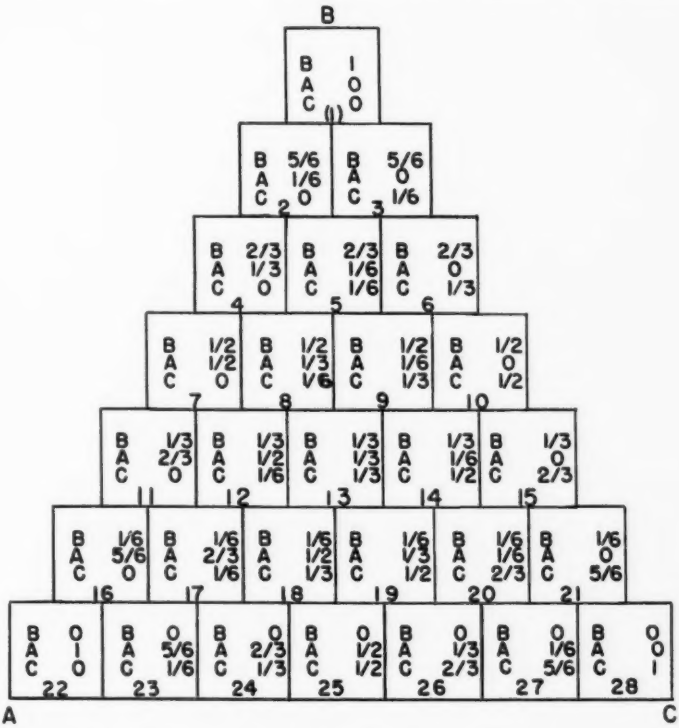

FIG. 1.—Triangle showing parts of solutions A, B, and C used in the twenty-eight different combinations. For example, square 13 contains one-third part solution B, one-third part solution A, and one-third part solution C.

solutions are practically constant, therefore these concentrations did not vary appreciably as the result of any mixture of the three solutions. In a similar manner a series of nutrients were made up in another triangle system, but in this case the cations varied and the anions remained constant. Solution D consisted of 0.0045 mol

$Mg(NO_3)_2$, 0.0045 mol $MgSO_4$, and 0.0045 mol $MgHPO_4$. Solution E consisted of 0.0045 mol KNO_3, 0.0045 mol K_2SO_4, and 0.0045 mol KH_2PO_4. Solution F consisted of 0.0060 mol $Ca(NO_3)_2$, 0.0060 mol $CaSO_4$, and 0.0060 mol $Ca(H_2PO_4)_2$. These solutions were utilized and mixed in a manner similar to that described for the first triangle, except that solutions D, E, and F were used in place of solutions A, B, and C respectively (fig. 1).

Biloxi soybeans were used as the final experimental material. The seeds were selected for uniformity, transplanted after germination, and watered, all as in the previous experiments. The pots were placed in the greenhouse at 75°–80° F. The relative humidity was generally above 60 per cent.

Three plants were placed in an 8×4-inch glazed jar containing quartz sand. Each set in the twenty-eight squares of the triangle contained ten jars for a total of thirty plants to each square. The sand was flushed with the various nutrient solutions daily and twice a week the sand was also flushed with distilled water. The plants in experiment I were planted October 1 and harvested October 21. The plants in experiment II were planted October 7 and harvested October 26.

At harvest the roots were washed free from sand and dipped into a saturated solution of sodium chloride to remove any particles adhering, then again washed and the plant divided into two fractions, (*a*) the tops, and (*b*) the roots and hypocotyls. Fresh weights were taken and the fractions were placed in a well ventilated drying oven at 80° C. for two days. Dry weights were then taken by means of a torsion balance sensitive to 0.01 gm. The data were recorded on a basis of 100 plants (figs. 2, 3).

Results

Experiment I

In this experiment the cations were constant throughout the series of solutions and the concentration of anions varied. Plants growing in the solution containing PO_4 as the only anion present (fig. 1, square 28) showed poor growth and evidences that the solution was toxic. The seedlings elongated at about the same rate as those in the other solutions, the cotyledons and first foliage leaves expanding

comparably. Soon after expanding, however, the primary leaves turned brown, and while still expanding the first two trifoliate leaves also developed discolored areas. The color change was from green to dark greenish brown, to light brown, to tan. The change was evident

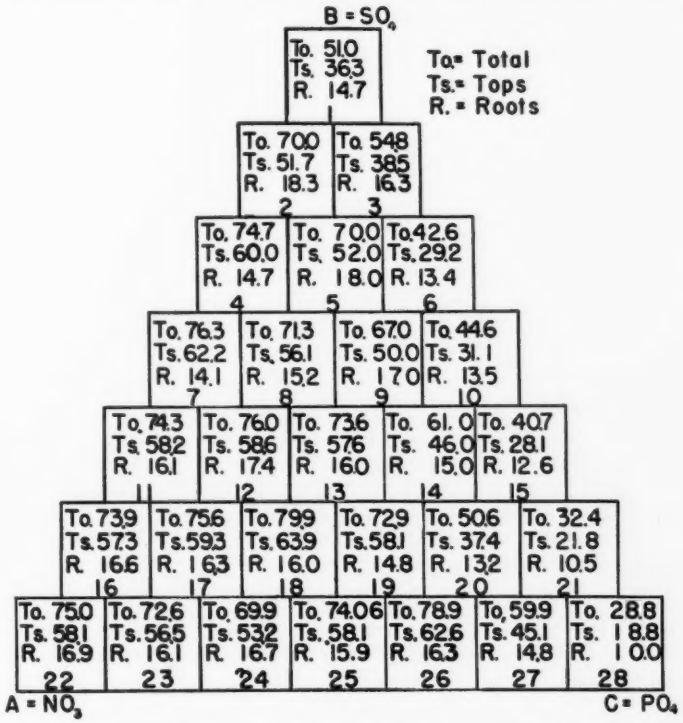

FIG. 2.—Dry weight accumulation per 100 plants of twenty-eight different solutions in which the cations remained constant and the anions varied.

first along the midribs and veins and then throughout the leaf, spreading from the veins. All the plants supplied with nutrient solutions deficient in nitrogen and containing phosphorus showed some evidences of these symptoms, and their amount and severity were in proportion to the amount of phosphorus present in the nutrient medium. Apparently these symptoms represent evidences of phosphorus toxicity. If small amounts of nitrates were present in the

nutrient solution, phosphorus toxicity was apparent only in those solutions containing comparatively high amounts of phosphorus. Thus no evidences of phosphorus toxicity were apparent in those solutions (fig. 1, squares 5, 13, 25) in which the values of both NO_3

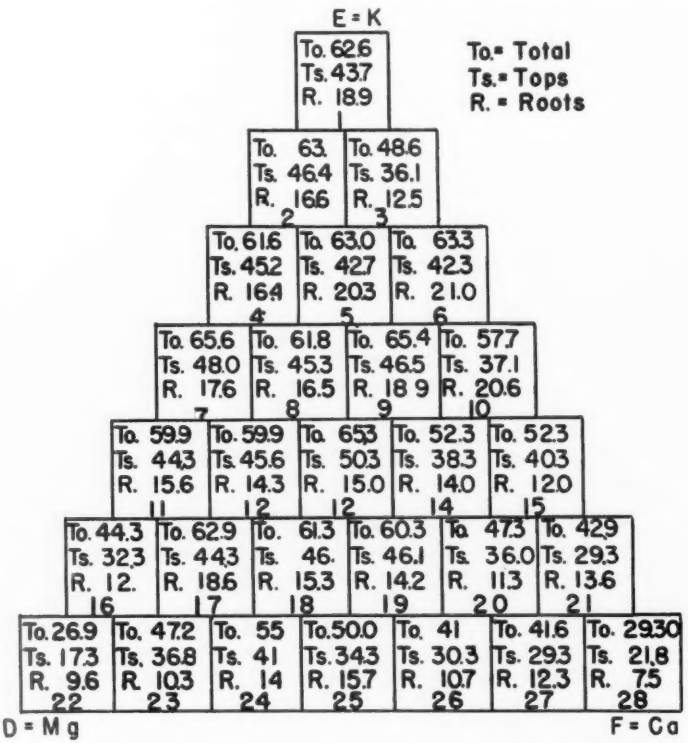

FIG. 3.—Dry weight accumulation per 100 plants of twenty-eight different solutions in which the anions remained constant and the cations varied.

and PO_4 were at one-sixth, one-third, or one-half their maximum. But in the solution in which the NO_3 was at one-third its maximum value and the PO_4 at two-thirds its maximum value, toxicity symptoms were apparent during early stages of growth (fig. 1, square 26). As the plants grew older these symptoms did not increase in severity and new growth seemed to be but slightly affected. In the solution containing one-sixth the maximum amount of NO_3 and one-third the

maximum amount of PO_4 (fig. 1, square 9) the symptoms developed much the same as just described, except that new growth continued to display a few discolored areas even up to the time of harvest. Thus there is a relationship between the relative concentration of nitrate and phosphate in the nutrient and the development of phosphate toxicity symptoms, a small amount of nitrate seemingly serving in some measure to counteract what otherwise might be a toxic concentration of phosphorus. The relationship between various concentrations of phosphate and nitrate ion in the accumulation of dry weight by the plants is shown in figure 2. In general, at any particular concentration of phosphorus, more dry weight was produced with increasing concentrations of nitrate.

When nitrate only was the major anion present (fig. 1, square 22), a rate and type of growth took place which compared favorably with the greatest growth in any of the other solutions, although the total dry weight was slightly lower than in a few of the other solutions (fig. 2, squares 7, 12, 18, 26). In no case did distinct symptoms of phosphorus or sulphur deficiency appear. This may have been because during germination and early growth, such as is represented by the period of these experiments, adequate supplies of these elements were available from storage supplies in the seed. It is also possible that, even though these elements were not in the nutrient solutions supplied to certain plants, these plants may have obtained some of them from the traces present in the sand but which was free from nitrogen and phosphorus. (The seedlings were germinated in sand and watered with tap water during the early stages of germination before transplanting.) There were no evidences of sulphate toxicity or deficiency at any time.

As the amount of PO_4 increased in the solution the importance of NO_3 for vegetative growth and accumulation of dry weight became much more evident (fig. 2). PO_4 may constitute as much as two-thirds the concentration of the anions present and yet active growth result if NO_3 is present as a third part (fig. 2, square 26).

Experiment II

Throughout the series in this experiment the anions were present at nearly the same concentration and the cations varied in relation to one another. At the time of harvest the plants showed a great

many differences between the various lots. Certain distinct characteristics varied in intensity from lot to lot. Thus some showed characteristic reddening of the petioles and midribs of the leaves, others reddish brown spots in the blades. While both these symptoms were apparent in some lots, others showed one and not the other. Because of this, certain symptoms which were characteristic of more than one lot were selected and their presence or absence recorded for each group. These patterns of response or symptoms are as follows, with the symbols used to designate them.

o Presence of red coloration:
- o In petiole of primary leaves
- oo In petiole of primary leaves and upper leaves
- ooo In petiole and midrib of primary leaves and upper leaves
- oooo In petiole and midrib of primary leaves and upper leaves and in the stem

\# Presence of reddish brown spots in blade of leaf (no relative degrees of this were noted; it was either present or absent)

= Presence of light brown areas in blade of leaf; these differed from the preceding in that the color was lighter and more widespread in the leaves

g Chlorosis:
- g Partial chlorosis of some leaves
- gg Chlorosis general
- ggg Chlorosis more general
- gggg Chlorosis extreme

The presence of these symptoms and the degree to which they were manifested are shown in figure 4. A comparison of the results indicates that the reddish coloration of petioles and midribs (symptom o) represents an effect of Ca toxicity, since it is present in all plants which receive relatively high concentrations of Ca, regardless of the relative amounts of the other ions The reddish brown spots in the blades (symptom #) represent evidence of Mg deficiency, since the symptom is present in all plants growing in magnesium-deficient solutions. The presence of light brown areas in the leaves (symptom =) represents evidence of calcium deficiency, since it is shown by all plants which grew in solutions deficient in calcium. It

is more difficult to interpret the chlorotic effects, but the presence of a high concentration of potassium seems to inhibit development of chlorosis, as does the presence of all three elements in a balanced condition. As for the growth response, the greatest correlation be-

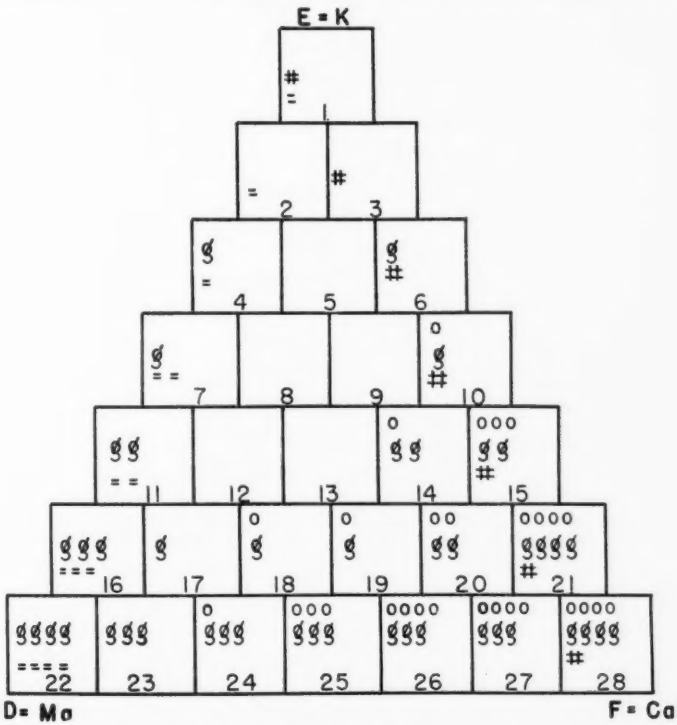

FIG. 4.—Toxicity and deficiency symptoms in twenty-eight different solutions in which the anions remained constant and the cations varied.

tween growth and variation in some ion is found in the case of variations in potassium. Thus high concentrations of potassium favor active vegetative growth even though both calcium and magnesium are very low in concentration in the solution (fig. 3).

On the basis of chemical analysis of plants, several investigators have shown that there exists a fairly definite relation between their phosphorus and nitrogen contents and the type of growth or stage

of development at the time of taking samples for analysis. The present investigation has further demonstrated that such a relation between nitrogen and phosphorus in the nutrient solution also affects the rate of growth of the plants and the appearance or lack of appearance of certain toxic symptoms. LEMMERMAN and BEHRENS (6) obtained satisfactory growth of oats in pots when the proportion between P_2O_5 and nitrogen was 1:1 but not when it was 3:1, in which case there was phosphate poisoning. DANIEL (3) found the nitrogen and phosphorus ratios within the plant tissue closely correlated. HUTCHINGS (5) found a close relationship in soybean between the phosphorus and nitrogen content of the plant material. BREAZEALE (1) stated that the absorption of nitrogen by wheat seedlings was not affected by the presence of either phosphorus or potassium; absorption of phosphorus was slightly increased by the presence of nitrogen. MOORE (7) found that the phosphate ion ordinarily used in nutrient solutions was injurious to peanut seedlings when grown in the light, but those grown in darkness were not subject to phosphate toxicity.

The relationship between phosphorus and nitrogen in soybean is important in overcoming toxicity of phosphorus. Phosphorus is toxic in very small concentrations if nitrogen is lacking, but it is not toxic even in fairly high concentrations if larger amounts of nitrogen are present. In general, one part of NO_3 will overcome the toxic effects of two parts of PO_4. In older plants this ratio may not be the same, seedlings being more susceptible to phosphate poisoning than older plants. This is possibly caused by the greater absorption of phosphorus at the seedling stage, the lower tolerance for phosphates, or the amount of phosphorus stored in the seeds. It is possible that the older plants have enough stored nitrogen to tolerate an outside ratio which under other circumstances would be toxic.

The writer wishes to express his appreciation of the helpful suggestions given by Dr. K. C. HAMNER during the course of these experiments.

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AVENA COLEOPTILE ASSAY OF ETHER EXTRACTS OF NODULES AND ROOTS OF BEAN, SOYBEAN, AND PEA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 511

GEORGE K. K. LINK AND VIRGINIA EGGERS

Introduction

The data and conclusions reported here have resulted from attempts at preliminary coleoptile assays of ether extracts of nodules and roots of red kidney bean (*Phaseolus vulgaris*), Biloxi soybean (*Soja max*), and Alaska pea (*Pisum sativum*). The study proceeded from the hypothesis that if growth substances, that is, auxones,[2] play the roles in growth indicated by current experiments and theory, then the auxone relations in galls will be anomalous in one or more respects as to kind, quantity, behavior, and other relations of the relevant auxones. It seemed most feasible to begin by limiting the inquiry to the auxin[3] fraction of root galls.

MOLLIARD (6) reported in 1912 that sterile filtrates of cultures of *Rhizobium radicicola* produced anomalous growth in roots of legumes. THIMANN (8) in 1936 reported that more auxin was diffused into agar from the young nodules than from the root tips of the garden pea, and that auxin applied to lateral root tips of the pea induced tumorous growth and inhibited elongation. He developed a theory to account for nodule formation in terms of the effects of auxin produced by the nodule bacteria on the cells of the invaded plant.

LINK, WILCOX, and LINK (5) in 1937, not aware of THIMANN'S paper, reported that strains of *Rhizobium phaseoli* in culture pro-

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[2] The concept auxone (5) includes all growth substances, that is, all active substances which effect or affect growth in the sense of increase in size, number, and morphologic difference of wholes and of their parts. For example, it includes auxins and auxin inhibitors, thiamine, and wound hormones. It is not a chemical, but a biologic concept.

[3] Auxin is here used to designate that class of auxones which are readily detectable by cell elongation of the *Avena* coleoptile.

duced auxins of the indoleacetic acid type, and that this acid, applied to roots of red kidney bean, produced bending, inhibition of elongation, and tumors, depending upon the concentration of the acid, the site of application to the root, and the age of the site. A theory was advanced which took note of the possibility that in gall growth the disturbed auxone relations (dysauxony) within the host probably involve: (*a*) auxones contributed by the foreign agent (heteroauxones); and (*b*) disturbances in the normal amounts, kinds, and behavior of auxones produced by the cells of the affected plant (autoauxones). LINK (**4**) reported that extracts of nodules of red kidney bean were more active in growth tests with bean and tomato seedlings and *Avena* coleoptile than extracts of the roots; that they contained more auxin of the indoleacetic acid type; and concluded that the hyperauxony of the nodules probably was largely due to indoleacetic acid contributed by the bacteria.

CHEN (**1**) in 1938 reported that *Rhizobium trifolii* produced indoleacetic acid in culture and that the most active cultures produced the greatest quantities. GEORGI and BEGUIN (**2**) in 1939 reported that four species of *Rhizobium* in culture produced indoleacetic acid. In two of these, the ineffective strains (in the sense of not fixing nitrogen) produced more indoleacetic acid than the effective strains. They reported also that *B. radiobacter*, which lives in nodules as a contaminant, is able to produce indoleacetic acid. They questioned whether indoleacetic acid plays a causal role in nodule growth. THIMANN and SCHNEIDER (**9**) in 1939 reported that nodules of red kidney bean are at least as effective in auxin production as coleoptile tips.

Investigation

Bean and soybean were grown in the garden in infected soil, and in the greenhouse in sterilized quartz sand watered with Shive's complete nutrient solution. The peas also were grown in the greenhouse in quartz; some inoculated, some not. The root systems were collected at different ages and at different times during the growing seasons of 1937–39. The root systems were thoroughly scrubbed and rinsed to rid them of soil and sand. The nodules were removed by hand with minimum injury to them and the roots. They were placed in ether, whole. The roots, both those from which nodules had been removed and those always free of them, were cut into pieces 1–3

inches long and then placed in ether. Preparation of ether and extraction was carried out according to VAN OVERBEEK's method (**10**). Extraction was continued for 22 hours. The ether extracts were evaporated to dryness and used the next or the second day in the *Avena* coleoptile test according to WENT's method (**12**). Each extract was taken up in 2 cc. of melted 3 per cent agar at 60° C. The

TABLE 1

ACTIVITY OF ETHER EXTRACTS OF NODULES AND ROOTS OF BEAN, SOYBEAN, AND PEA EXPRESSED IN DEGREES OF CURVATURE OF AVENA COLEOPTILES

PLANT	MATERIAL	SAMPLE (GM.)	AVERAGE CURVATURES OF TWELVE COLEOPTILES PER TEST (IN DEGREES)			
			FIRST DILUTION	SECOND DILUTION	THIRD DILUTION	FOURTH DILUTION
Bean	Roots (st)*	40	9.9	9.2	7.9	.....
	Roots (d-n)†	8	19.2	6.6	2.0	.....
	Nodules‡	8	18.0	19.0	23.4	21.0
Soybean	Roots (st)	40	12.6	12.2	8.4	.....
	Roots (d-n)	8	20.2	13.5	8.3	.....
	Nodules	8	18.7	16.2	10.3	.....
Pea	Roots (st)	40	19.3	21.7	9.9	.....
	Roots (d-n)	5	21.2	10.6	8.4	.....
	Nodules	5	17.6	28.3	22.1	.....
Indoleacetic acid			10γ 3.7 20γ 5.0 40γ 12.3 60γ 19.0		Each value is average of four tests, each with twelve coleoptiles per concentration	

* Roots (st) = roots grown in sterilized quartz.
† Roots (d-n) = roots denodulated. ‡ Nodules from denodulated sample of roots.

mixture was thoroughly stirred and then allowed to stand for an hour, after which it was remelted. A half cc. and a 0.75 cc. portion of the mixture were diluted with 0.5 cc. and 0.75 cc. of 3 per cent agar respectively. These dilutions were allowed to stand for an hour and upon remelting were poured into a mold, 8×10×1.5 mm. Each plate was cut into twelve blocks of equal volume. The blocks were applied to coleoptiles twice decapitated. For each dilution twelve test plants were used. In order that the results might be comparable for tests of different days, controls of indoleacetic acid in agar in concentrations of 10, 20, 40, and 60 gamma per liter were

run with twelve coleoptiles per test. The curvatures were recorded after 90 minutes. The data are given in table 1.

Several experiments were conducted to determine the kinds of auxins and their relative proportion in extracts of nodules and roots of the red kidney bean. To this end VAN OVERBEEK's adaptation (11) of the KÖGL, HAAGEN-SMIT, and ERXLEBEN procedure was

TABLE 2

ACTIVITY OF NON-REFLUXED AND REFLUXED ETHER EXTRACTS OF NODULES AND ROOTS OF BEAN, EXPRESSED IN DEGREES CURVATURE OF AVENA COLEOPTILES

TREATMENT	MATERIAL	SAMPLE (GM.)	AVERAGE CURVATURE OF TWELVE COLEOPTILES PER TEST (IN DEGREES)		
			FIRST DILUTION	SECOND DILUTION	THIRD DILUTION
Not refluxed	Roots	8	19.2	6.6	2.0
	Nodules	8	18.0	19.0	23.4
H_2O reflux	Roots	8	Sample lost	.....	.....
	Nodules	8	15.0	19.5	20.6
5% HCl reflux	Roots	8	19.6	3.6	3.6
	Nodules	8	23.0	21.7	19.0
0.5 N NaOH reflux	Roots	8	4.8	0.0	2.6
	Nodules	8	15.0	16.0	14.5
Indoleacetic acid		10γ	3.7		Each value is average of four tests each with twelve coleoptiles per concentration
		20γ	5.0		
		40γ	12.3		
		60γ	19.0		

used. The procedure is based on the observations that: (*a*) indoleacetic acid is heat-stable to alkali but not to acid; (*b*) auxin *a* is heat-stable to acid but not to alkali; and (*c*) auxin *b* is heat-stable to neither acid nor alkali. Forty gm. samples of nodules and of nodule-freed roots of bean were extracted with ether for 22 hours. After extraction and partial evaporation, the ether extracts were divided each into five equal portions. One pair of these portions (one of nodule extract and one of root extract) was evaporated to dryness without further treatment; a second pair was refluxed over the steam bath for fifteen minutes with 3 cc. of H_2O, adjusted as to pH (slightly

acid), taken up in ether and the ether fraction evaporated to dryness; a third pair was refluxed each with 3 cc. of 5 per cent HCl; and the fourth each with 3 cc. of 0.5 N NaOH. Each was adjusted as to pH (slightly acid), taken up in ether and the ether fraction evaporated to dryness. The residues of the eight samples were taken up in agar and tested. The data are recorded in table 2.

Discussion

The outstanding features of the data of table 1 are: lack of linear proportionality between dilution and activity of most extracts; rise of activity values through one or several dilutions of the nodule extracts of bean and pea and of the sterile pea roots; difference in activity and dilution values of the extracts of the nodules of bean and pea compared with those of root extracts; and differences between activity values of the extracts of sterile roots and of denodulated roots.

Great care was taken to insure equal distribution of the extracts during the initial mixing with agar and in subsequent dilutions. The variations in curvature within the sets of plants tested with the first dilutions did not indicate that the lack of proportionality between dilution and activity was due to uneven distribution. It is possible that this lack of proportionality, especially of the nodule extracts, was due to supramaximal activity of the extracts in the lower dilutions. The relative magnitude of the values for the different dilutions, however, indicates that this is not an adequate explanation. It is more likely that in great part the lack of proportionality was due to change in activity of the extract during the process of mixing and dilution with agar. Phenomena noted by others and reviewed and analyzed by GOODWIN (3) and STEWART (7) possibly are responsible for the effects. The assumption that we were in part dealing with substances which do not induce or which inhibit cell elongation—but change upon hydrolysis into substances which effect cell elongation—aids a rational interpretation of the observed rise of activity of the extracts with increasing dilution. If this interpretation is correct, then the activity values recorded are not indices of auxin content of the extracts but of positive auxin balances, in the sense that the net result of the antagonistic effects of the components of the extracts on the coleoptile is cell elongation.

Since we have not yet succeeded in determining experimentally the validity of one or all of these explanations, the data are presented in their present far from satisfactory form. The order of differences recorded has appeared in many tests not included here. Because we were primarily interested in comparative values of the nodule and root extracts, these were tested first each day. As a result the indoleacetic tests, in order of decreasing concentration, came at the time of declining sensitivity of the coleoptiles. This is responsible for the disproportionately low values of the higher concentrations of indoleacetic acid relative to the lower, and to the values of the nodule and root extracts. For these reasons, in part, no attempt has been made to use the indoleacetic acid values in calculating the auxin contents of the nodules and root samples.

If the activity values of all dilutions—and especially those of the second and third—are considered, the conclusion seems warranted that either the auxin content or the positive auxin balance is greater in the nodule extracts than in the extracts of denodulated roots, and that either the auxin content or the positive balance of these, in turn, is greater than that of the extracts from roots grown in sterilized sand. The same order of differences is indicated for the extracts of the nodules, denodulated roots, and sterile roots of soybean. In the soybean the small difference between the activities of the extracts of the nodules and of the denodulated roots may be due to less complete extraction of the nodules than of the roots. Relative to the bean and pea extracts, the difference may be due to the fact that the soybean nodules were larger, smoother, and firmer than the nodules of bean and pea. The wounds made in picking them were comparatively much smaller. Hence without grinding and repeated extraction to break possible conjugate unions between auxin, auxin precursor, and other cell constituents, extraction of the soybean nodules probably was the least complete of any extraction made. The activity of the extracts of sterile soybean roots (40 gm.) relative to that of the denodulated roots (8 gm.), like that of the bean and pea roots, is decidedly lower.

The greater increase in activities with dilution in the nodule extracts of bean and pea, compared with the denodulated root extracts, may indicate that the composition of these extracts is not the same. If we assume that the extracts contain auxin inhibitors (precursors),

then the data may indicate that the nodule extracts are richer in these than the extracts of the denodulated roots, and that the denodulated roots are richer in these than the sterile roots.

The activity of the nodule extracts of bean after refluxing with NaOH and HCl was greater than that of the denodulated root extract, and the activity of the nodule extracts after acid hydrolysis was greater than after alkali hydrolysis. In accordance with VAN OVERBEEK'S interpretation (11), we inferred from such data of earlier experiments that the nodule extract and root extract contained auxin *a* and indoleacetic acid, and that the nodules contained more of each, and especially more of indoleacetic acid. In the light of STEWART'S findings (7) it is not impossible that the activity noted after alkali hydrolysis is not an index of presence and quantity of indoleacetic, but in part of auxin derived by hydrolysis from an auxin inhibitor or precursor. The auxin activity noted after acid hydrolysis may indicate that the roots and nodules of the bean contain something that behaves as auxin *a* in hydrolysis.

In short, the data indicate that the nodules of bean, soybean, and pea have greater and different auxone contents than the roots which bear them, and that these in turn have greater and different auxone contents than the roots when grown in *Rhizobium*-free substrates. The findings favor the hypothesis that the auxone relations in galls are anomalous.

Summary

1. Ether extracts of nodules of bean and pea were more active in effecting negative curvatures of coleoptiles in the *Avena* test than those of denodulated roots. These in turn were more active than those of roots grown in sterilized quartz sand.

2. The same relations, but less marked, were found for nodules and roots of soybean.

3. Negative bending activity of extracts of nodules of bean and pea and of sterile pea roots was increased by agar dilution. This may indicate a fraction in each extract which changes to auxin by hydrolysis, and that the activity noted is a measure of a positive auxin balance between fractions antagonistic in their effect on cell elongation in the coleoptile. The dilution effect was most pronounced in extracts of nodules of bean and pea.

4. Extracts of nodules and of roots of bean contained a fraction

which behaved like auxin *a* in acid hydrolysis. The nodule extract contained more of it than the root extract.

5. Extracts of nodules and of roots of bean contained a fraction which was auxinic after alkali hydrolysis. The nodule extract contained more of it than the root extract. This fraction may be composed of auxin present before hydrolysis which behaves like indoleacetic acid in alkali hydrolysis, of auxin derived from a precursor by hydrolysis, or of both.

6. It is inferred that the nodules of bean, soybean, and pea have greater and different auxone contents than the roots which bear them, and that these in turn have greater and different auxone contents than the roots when grown in sterilized substrates.

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INTERRELATION OF LIGHT AND DARKNESS IN PHOTOPERIODIC INDUCTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 512

KARL C. HAMNER[1]

(WITH FIVE FIGURES)

Introduction

Much of the work on photoperiodism has been based on the use of 24-hour cycles, some portion of which constituted a photoperiod and the remainder a dark period. Thus any increase in the length of the photoperiod was accompanied by a corresponding decrease in the length of the dark period, or vice versa, the total length of the cycle remaining unchanged. The resulting responses were ascribed (1) to the relative extent of the photoperiod, (2) to the relative duration of the dark period, or (3) to both of these periods in combination. There has been a tendency to adhere to one or the other of the first two suggestions more than to the third. Experiments on vernalization taken in conjunction with those on photoperiodism have also received wide attention (12).

In the present investigation the possible interrelationships of reactions which result from exposure to light and reactions which result from exposure to darkness have been emphasized. Plants were chosen which are known to be very sensitive to photoperiod and which show little modification of their photoperiodic response over relatively wide ranges of temperature. No specific investigation of plants known to be thermally limited in their photoperiodic response was undertaken. Cocklebur and Biloxi soybean are generally considered short-day plants and dill and beet are classified as long-day plants. The variety of beet used, differing from many others, is not narrowly thermally limited and behaves as an annual plant. The seed was obtained from Dr. E. Carsner.

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Procedure and results

The experiments recorded here were carried out during the late spring, summer, and early fall. The procedure of experimentation, the precautions employed, and the equipment used were similar to those described previously (**5**).

Work with Biloxi soybean (*Soja max*)

Seeds of Biloxi soybean were planted in $4\frac{1}{2}$-inch clay pots containing an open garden soil. Four seeds were planted in each pot and the pots were placed on a well-lighted bench in the greenhouse. The plants were exposed to the natural daylight prevailing and supplemented from sunset until 2:00 A.M. with artificial light from Mazda filament lamps of about 100 foot-candles' intensity at the leaf surface. The photoperiod thus varied from $18\frac{1}{2}$ to $20\frac{1}{2}$ hours. After the seedlings had emerged from the soil and the cotyledons of most of them were fully expanded, two were removed from each pot, an attempt being made to secure maximum uniformity. The plants were well watered and grew vigorously throughout the experimental period. At the time the third trifoliate leaf was expanding they were employed for the detailed experiments.

Since it is known that Biloxi soybean requires a succession of relatively short photoperiods each followed by a relatively long dark period for floral induction, the relationship between length of the dark period and length of the light period was studied. Much of this work involved the use of cycles other than 24 hours' duration and so artificial light as well as daylight was used. In most of the experiments a number of vegetative plants were taken from the greenhouse at 5:00 P.M. and placed in a temperature-controlled room in which was installed an Eveready Carbon arc, burning sunshine carbons (**8**). The arc light burned continuously and furnished a quality of light similar to natural sunlight at an intensity of about 1200 foot-candles at the leaf surface. All the plants were maintained under the arc light from 5:00 P.M. until 8:00 A.M. the next morning. Including both the daylight and the arc light, therefore, all received a photoperiod of at least 26 hours at the time the different groups were subjected to the first dark period of any particular treatment. Each group was maintained subsequently at a dark period of any desired

length by placing the plants in a darkroom where they remained for the desired length of time. They were then returned to the room lighted by the arc where they were subjected to the various photoperiods. The temperature in the lighted room varied from 73° to 78° F. and in the darkroom from 75° to 80° F. The relative humidity in the two rooms varied between 50 and 80 per cent. Biloxi soybeans were employed in these experiments.

EXPERIMENT 1.—Fifty-five pots, each containing two uniform plants, were divided into eleven equal lots at 8:00 A.M., after having received the initial period of illumination. Each lot then received a 16-hour dark period. Individual lots were next exposed to the following photoperiods: 10, 11, 12, 13, 14, 16, 18, 20, 24, 30, and 36 hours. After any given lot was exposed to this photoperiod it was returned to the darkroom where it remained for 16 hours. It was then again exposed to a photoperiod equal in duration to the one it had previously received. At the end of seven cycles of such treatment each lot was maintained in the room illuminated by the arc light until 7:00 A.M. the following morning, at which time the pots were removed to the greenhouse bench and maintained at conditions of long photoperiod. Thus each treatment was ended by a long photoperiod which varied somewhat in length for the various lots, depending upon the time of day or night the treatment terminated.

EXPERIMENT 2.—Fifty-five pots of plants, selected as before, were placed in the arc-light room from 5:00 P.M. until 8:00 A.M., at which time they were divided into eleven lots. Individual lots then received the following dark periods: 8, $8\frac{1}{2}$, 9, $9\frac{1}{2}$, 10, $10\frac{1}{2}$, 11, $11\frac{1}{2}$, 12, 14, and 16 hours. Subsequent to the dark period, each lot received a 16-hour photoperiod and then was again subjected to its respective dark period. These treatments were continued until each lot had received seven cycles of treatment, subsequent to which they were treated with a period in the arc-light room as before, and then placed under long photoperiod conditions in the greenhouse.

EXPERIMENT 3.—Seventy pots of plants, selected as before, were placed in the arc-light room from 5:00 P.M. until 8:00 A.M., and then divided into fourteen lots. Individual lots then received the following dark periods: 4, 6, $6\frac{1}{2}$, 7, $7\frac{1}{2}$, 8, $8\frac{1}{2}$, 9, $9\frac{1}{2}$, 10, 11, 12, 14, and 20 hours. Subsequent to the dark period, each lot received a 4-hour photoperiod and then was again subjected to its respective dark period.

These treatments were continued until each lot had received seven cycles of treatment, subsequent to which they were illuminated for a

TABLE 1

EFFECTIVENESS OF VARIOUS CYCLES IN INDUCING FLORAL BUD DIFFERENTIATION IN BILOXI SOYBEAN (TEN PLANTS IN EACH LOT)

SEVEN CYCLES, EACH CONSISTING OF A 16-HOUR DARK PERIOD ACCOMPANIED BY FOLLOWING PHOTOPERIODS

	LENGTH OF PHOTOPERIOD IN HOURS										
	10	11	12	13	14	16	18	20	24	30	36
No. of plants possessing floral primordia or flowers	10	9	9	8	8	4	2	0	0	0	0
No. of nodes possessing floral primordia or flowers	18	19	17	12	13	7	2	0	0	0	0

SEVEN CYCLES, EACH CONSISTING OF A 16-HOUR PHOTOPERIOD ACCOMPANIED BY FOLLOWING DARK PERIODS

	LENGTH OF DARK PERIOD IN HOURS									
	8	8½	9	9½	10	10½	11	11½	12	14	16
No. of plants possessing floral primordia or flowers	0	0	0	0	0	2	3	4	5	4	5
No. of nodes possessing floral primordia or flowers	0	0	0	0	0	2	5	6	7	7	8

SEVEN CYCLES, EACH CONSISTING OF A 4-HOUR PHOTOPERIOD ACCOMPANIED BY FOLLOWING DARK PERIODS

	LENGTH OF DARK PERIOD IN HOURS													
	4	6	6½	7	7½	8	8½	9	9½	10	11	12	14	20
No. of plants possessing floral primordia or flowers	0	0	0	0	0	0	0	0	0	0	2	3	3	4
No. of nodes possessing floral primordia or flowers	0	0	0	0	0	0	0	0	0	0	3	5	4	5

period in the room with the arc light as before and then placed under conditions of long photoperiod in the greenhouse.

In each experiment, at the close of the specific treatment to which

any particular lot was exposed, the plants were allowed to develop on the greenhouse bench on a photoperiod in excess of 18 hours for 3–4 weeks. The plants were then carefully dissected and the presence or absence of flowers at each node on the main axis noted.

The results (table 1; fig. 1) indicate that, regardless of the length of the photoperiod, the plants will not flower unless they receive dark periods in excess of 10 hours. If the plants receive a succession of seven cycles they may flower or not, depending upon the conditions prevailing during the photoperiods of these cycles (see later). Under

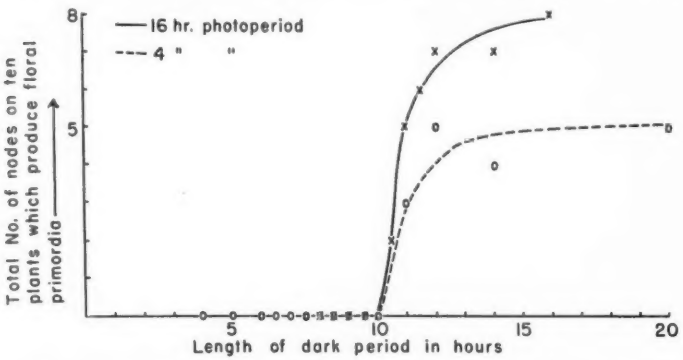

FIG. 1.—Effect of length of dark period of photoinductive cycle upon number of floral primordia produced by Biloxi soybean. All plants received seven cycles, each consisting of a 16-hour or a 4-hour light period and a dark period as indicated.

the conditions of the preceding three experiments, Biloxi soybean failed to initiate floral primordia on any cycle which did not include dark periods of more than 10 hours.

Figure 1 shows that the length of the dark period which is critical does not vary appreciably, whether the accompanying photoperiods are relatively long or short. While some floral initiation may occur as the result of exposure to a $10\frac{1}{2}$-hour dark period, a 12-hour dark period seems to be more effective. A 20-hour dark period, on the other hand, is neither more nor less effective than a 12-hour dark period; plants which received seven 16-hour cycles consisting of 4-hour light and 12-hour dark periods produced as many floral primordia as did plants which received seven 24-hour cycles consisting

of 4-hour light and 20-hour dark periods. This was true in spite of the fact that, at the end of the treatment, the latter plants were much smaller and the leaves were yellower than the former. Thus floral initiation may be limited by dark periods shorter than 12 hours and completely inhibited by dark periods shorter than 10 hours.

While the length of the dark period may determine qualitatively whether or not the plants will initiate floral primordia as the result of a particular treatment, the quantitative response or the number of floral primordia actually initiated is apparently determined to a

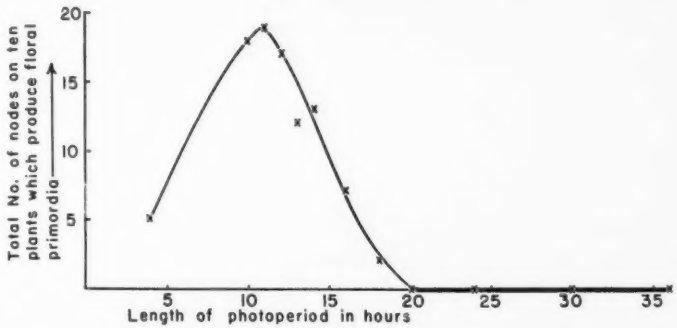

FIG. 2.—Effect of length of photoperiod upon number of floral primordia produced by Biloxi soybean. All plants received seven cycles, each consisting of a 16-hour dark period and a period of light as indicated.

marked degree by the duration of the photoperiod (fig. 2). The optimum length of photoperiod seems to be between 10 and 12 hours at a given intensity and temperature (1200 foot-candles and 73°–78° F.), and photoperiods either longer or shorter than this optimum result in the differentiation of a decreased number of floral primordia. The maximum photoperiod favorable for the initiation of floral primordia in any of the cycles used was between 18 and 20 hours and the minimum was below 4 hours.

Since these results were obtained with plants exposed to artificially controlled cycles of light and darkness, experiments were designed to compare the results with the responses of Biloxi soybean plants exposed in various ways to differing photoperiods in the greenhouse under natural sunlight.

EXPERIMENT 4.—Thirty-five pots of plants were placed on a truck which remained in the greenhouse under bright sunlight for 9 hours

TABLE 2

RESPONSES OF BILOXI SOYBEAN TO VARIOUS CYCLES, EACH CONSISTING OF 9 HOURS OF NATURAL LIGHT ACCOMPANIED BY 15 HOURS OF DARKNESS (TEN PLANTS IN EACH LOT)

	No. of cycles of treatment						
	1	2	3	4	5	6	7
No. of plants possessing floral primordia or flowers	0	5	10	10	10	10	10
No. of nodes possessing floral primordia or flowers	0	7	19	24	37	47	56

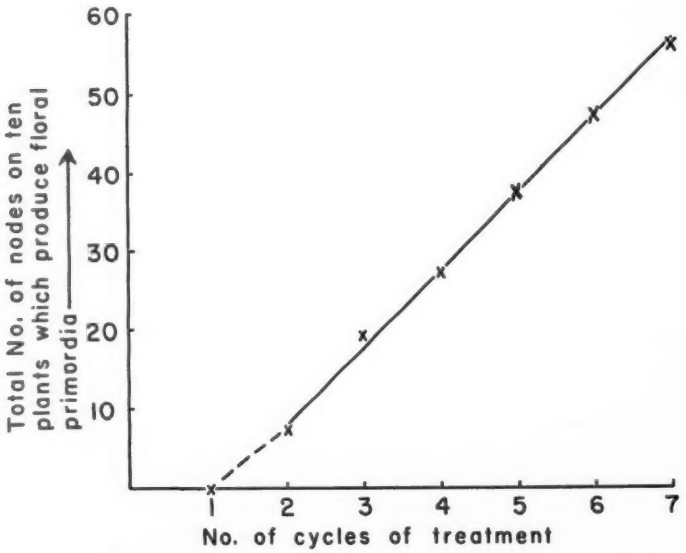

FIG. 3.—Effect of number of cycles, each consisting of a 9-hour photoperiod and a 15-hour dark period, on number of floral primordia produced by Biloxi soybean.

each day and in complete darkness for 15 hours each day. Five pots (ten plants) were removed from the truck after one such cycle of short photoperiod and long dark period, five more after two, and so

on up to seven cycles until all the plants had been removed. After treatment, the plants were returned to the greenhouse bench under long photoperiod and allowed to develop until harvested 4 weeks later. This experiment was carried out concurrently with experiments 1–3 (table 2; fig. 3).

EXPERIMENT 5.—Seventy pots of plants were placed on seven trucks, ten pots per truck, and the trucks placed in bright sunlight for 9 hours each day and then taken into separate light-tight compartments. In each compartment were suspended Mazda filament lamps controlled by time clocks, so that in addition to the 9 hours of natural light the plants on each truck could be exposed to supplementary illumination of any desired duration. Separate lots of twenty plants were each exposed repeatedly to cycles consisting of the following hours of light and dark:

Hours of light	Hours of dark	Hours of light	Hours of dark
13	11	15½	8½
14	10	16	8
14½	9½	16½	7½
15	9		

The results of this experiment are recorded in table 3.

TABLE 3

RESPONSE OF BILOXI SOYBEAN TO THIRTY CYCLES, EACH CONSISTING OF A PHOTOPERIOD AND A DARK PERIOD AS INDICATED (TWENTY PLANTS IN EACH LOT)

CHARACTER OF CYCLE	NO. OF PLANTS PRODUCING FLORAL PRIMORDIA OR FLOWERS
16½-hour photoperiod and 7½-hour dark period	0
16-hour photoperiod and 8-hour dark period	0
15½-hour photoperiod and 8½-hour dark period	0
15-hour photoperiod and 9-hour dark period	0
14½-hour photoperiod and 9½-hour dark period	0
14-hour photoperiod and 10-hour dark period	14
13-hour photoperiod and 11-hour dark period	20

Under greenhouse conditions, with bright sunlight during a portion of the photoperiod, the plants responded more rapidly than under the artificially controlled conditions of those previously discussed. Thus the ten plants which received seven cycles each consisting of 9 hours of natural light and 15 hours of darkness produced floral primordia at a total of fifty-six nodes as compared with those

plants under artificially controlled cycles of 10 hours of light and 16 hours of dark, which had floral primordia or flowers at a total of only eighteen nodes. While the total length of the artificially controlled cycle was 2 hours longer than the natural cycle, comparison seems justifiable.

The greater effectiveness of the photoperiodic treatments in the greenhouse in bringing about initiation of a greater number of floral primordia may have been associated with the fact that the temperatures there were uniformly higher than in the controlled environment; they may have been associated with the fact that the plants in the greenhouse were exposed to much higher intensities of light during their photoperiods; or there may have been other factors. It seemed most likely that light intensity was the most important factor, and a series of experiments were carried out in relation to the effect of duration and intensity of light during a short photoperiod.

EXPERIMENT 6.—One hundred and twenty vegetative plants (sixty pots) were removed from the greenhouse at 5:00 P.M. and divided into two groups of thirty pots each. One group was placed in a large darkroom and the plants arranged in six lots, each plant of any particular lot at the same distance from a 1000-watt Mazda lamp. The distances of the various lots from the lamp were such that, when the lamp was burning, respective lots received 50, 100, 150, 200, 400, and 800 foot-candles of illumination. The other group of thirty pots was placed in an adjoining darkroom so connected with the other that essentially the same temperature prevailed in both rooms at all times. The plants in this room were divided into lots and arranged in relation to a Mazda lamp as were the plants of the first room. In one room the lamp burned for 5 hours out of each 24 and in the other room for 10 hours out of each 24. The treatment was continued for 7 days, or until all plants had undergone seven cycles in the experimental room. The temperature in the experimental rooms varied somewhat, ranging from 75° to 100° F., usually being slightly higher during the photoperiod than during the dark period. After treatment, the plants were returned to the greenhouse and placed under long photoperiod, where they remained for 4 weeks, at which time they were dissected and the presence or absence of floral buds at each node noted (table 4; fig. 4).

Whether the plants were exposed to 5-hour or 10-hour photoperiods, the number of nodes possessing floral primordia or flowers

TABLE 4

EFFECT OF DURATION AND INTENSITY OF LIGHT DURING SEVEN CYCLES OF SHORT PHOTOPERIOD ON INITIATION OF FLOWERS IN BILOXI SOYBEAN

LIGHT INTENSITY DURING EACH OF SEVEN CYCLES (FOOT-CANDLES)	NO. OF PLANTS IN EACH TREATMENT	NO. OF PLANTS WITH FLOWER BUDS IN THE VARIOUS TREATMENTS		NO. OF NODES WITH FLOWER BUDS ON TOTAL OF TEN PLANTS	
		5-HOUR PHOTOPERIOD	10-HOUR PHOTOPERIOD	5-HOUR PHOTOPERIOD	10-HOUR PHOTOPERIOD
50	10	0	0	0	0
100	10	0	3	0	3
150	10	2	10	4	14
200	10	4	10	6	19
400	10	10	10	13	29
800	10	10	10	31	37

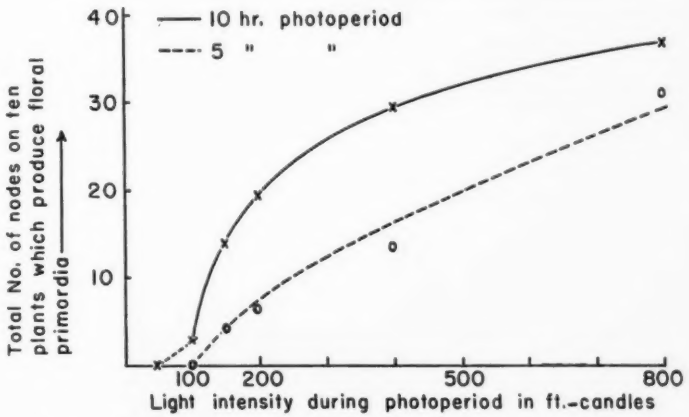

FIG. 4.—Effect of duration and intensity of light during seven photoinductive cycles on subsequent initiation of floral primordia by Biloxi soybean.

increased in proportion to the intensity of light during the photoperiod. The intensities used throughout these experiments were relatively low, and in the case of the lowest intensity used, 50 foot-candles, no flowers were produced at either photoperiod. The dura-

tion and intensity of light during the photoperiod produce a marked effect upon the number of floral primordia formed. Thus at any given intensity the number of floral primordia formed on the plant exposed to cycles with 5-hour photoperiods was approximately one-half the number produced at a comparable intensity on plants exposed to cycles with 10-hour photoperiods. Those plants which received 10-hour photoperiods developed more floral primordia at the higher intensities of light than did the plants which received a 5-hour photoperiod. From the results it would appear that any given intensity during a 5-hour photoperiod brought about a response in the plants comparable with the response brought about by one-half that intensity during a 10-hour photoperiod. It appears probable that under these conditions of relatively short photoperiod, low light intensity, long dark period, and relatively high temperature, floral initiation is roughly proportional to duration and intensity of light.

Work on the cocklebur (*Xanthium pennsylvanicum*)

Experiments with Biloxi soybean have shown that photoperiodic induction is brought about by reactions taking place during exposure to light operating in conjunction with reactions taking place during exposure to darkness. Under certain conditions the number of flowers produced may be roughly proportional to the intensity and duration of the light to which the plants are exposed. Under a variety of conditions, *Xanthium* may be induced to flower as the result of an exposure to one long dark period (**5**). Thus it is difficult to differentiate between the effects on floral initiation of previous photoperiods to which *Xanthium* plants have been exposed in bringing them to an age suitable for experimentation and the effects of the experimental treatment which subsequently result in their flowering.

It has been found repeatedly that *Xanthium* will not initiate floral primordia on any cycle in which the dark period is not at least more than $8\frac{1}{2}$–9 hours. Preliminary experiments with repeated exposures to cycles which consisted of as little as 3 minutes of light followed by 3 hours of darkness indicated that a condition might be reached such that the plants would not flower, even if subsequently exposed to a long dark period. To test out this possibility, a series of experiments were conducted.

EXPERIMENT 7.—One hundred thirty pots of vegetative *Xanthium* plants which had been growing on long photoperiod in the greenhouse for 4 weeks were removed from the greenhouse at 5:00 P.M. after a bright warm day and placed in a darkroom. Ten of the plants, serving as initial controls, were placed in a darkroom where they received no light whatsoever for 12 hours. The remaining 120 plants were placed in another darkroom in which were suspended Mazda lamps controlled by time clocks. Every 3 hours the plants in this room were exposed to light from these lamps for 3 minutes. The light intensity during this exposure was about 200 foot-candles at the leaf surface and the room temperature varied from 76° to 83° F. The

TABLE 5

EFFECT OF NUMBER OF CYCLES, EACH CONSISTING OF 3 MINUTES OF LIGHT AND 3 HOURS OF DARKNESS, ON FLORAL INITIATION IN XANTHIUM WHEN ALL PLANTS IMMEDIATELY SUBSEQUENT TO TREATMENT WITH SHORT CYCLES RECEIVED 12 HOURS OF COMPLETE DARKNESS (TEN PLANTS IN EACH LOT)

	No. of cycles of treatment											
	0	1	2	3	4	5	6	7	8	9	10	11	12
Flowering condition after 3 weeks on long photoperiod in greenhouse	10 fl. pr.*	6 fl. pr., 4 infl. pr.	4 fl. pr., 6 veg.	veg.	veg.	veg.	veg.	veg.	veg.	veg.	veg.	veg.	veg.

* In this and subsequent tables dealing with *Xanthium*, the condition of the terminal bud at time of dissection is indicated as follows:

veg.: strictly vegetative
infl. pr.: inflorescence primordia
fl. pr.: flower primordia
mac. fl. & fr.: macroscopic flowers and fruits

plants were thus exposed to very short cycles consisting of 3 hours of darkness and 3 minutes of light. At the end of each of the twelve cycles of treatment in this room, ten plants were removed. The treatments continued for 36 hours, at which time the last ten plants were removed. Upon removal from the room, each lot of plants was exposed to 12 hours of continuous darkness in another darkroom, following which they were returned to long photoperiod in the greenhouse; or, if the treatment terminated at night, they were placed under the arc light in the room previously discussed under the experiments on soybean, where they remained until 7:00 A.M. the next morning, at which time they were removed to the greenhouse bench

alongside the other plants and continued on the conditions of long photoperiod.

All plants remained in the greenhouse for 3 weeks, after which they were carefully dissected and the presence or absence of floral buds noted (table 5).

Xanthium plants which have been exposed to natural light on a bright day in the greenhouse will be photoperiodically induced if they are removed at 5:00 P.M. and immediately exposed to a 12-hour dark period. On the other hand, comparable plants will not be photoperiodically induced if the exposure to the 12-hour dark period is preceded by exposure to four or more cycles each consisting of 3 minutes of light and 3 hours of darkness. These results indicate that photoperiodic induction in *Xanthium* results not only from the exposure to a long dark period but is also affected by the nature of the photoperiod which precedes it.

EXPERIMENT 8.—Since from these experiments the character of a period of light as well as a dark period seemed to influence photoperiodic induction in *Xanthium*, an attempt was made to determine what effect the duration and intensity of the light period which immediately precedes the long dark period would have. Eighty vegetative plants were removed from the greenhouse at 5:00 P.M. and treated with twelve consecutive cycles consisting of 3 hours of darkness and 3 minutes of light. The plants were then divided into eight lots of ten plants each. One lot, to serve as controls, was immediately exposed to a 12-hour dark period and then placed in the room illuminated by the arc light until the next morning, when the plants were placed and maintained on long photoperiod in the greenhouse. Five other lots were placed in bright sunlight in the greenhouse, one lot continuing there for 2 hours, one for 4, one for 6, one for 8, and one for 10. Subsequent to their respective photoperiods in the greenhouse each of the five lots was exposed to darkness for 12 hours, transferred to the arc-light room until 7:00 A.M. the following morning, and then returned to conditions of long photoperiod in the greenhouse. The remaining two lots were placed in a room at a temperature of approximately 80° F., where they were exposed to light from a Mazda lamp for 10 hours. One lot received 10 foot-candles of light and another received 100 foot-candles at the leaf surface. Subsequent to this 10-hour photoperiod each of these two lots was ex-

posed to a 12-hour dark period, transferred to the arc-light room until 7:00 A.M. the next morning, and then returned to conditions of long photoperiod in the greenhouse. The plants of all the experiments remained in the greenhouse for 3 weeks, where they received a photoperiod of 18–20 hours and a dark period of 4–6 hours during each 24-hour day. At the end of 3 weeks all plants were carefully dissected (table 6).

From the results of these two experiments it seems obvious that there is an interaction or interrelation between light and darkness in bringing about photoperiodic induction in *Xanthium*, an interrela-

TABLE 6

EFFECT OF VARYING LENGTHS AND INTENSITIES OF PHOTOPERIOD ON FLORAL INITIATION OF XANTHIUM PLANTS PREVIOUSLY CONDITIONED BY TREATMENT WITH TWELVE CYCLES, EACH CONSISTING OF 3-MINUTE LIGHT PERIOD AND 3-HOUR DARK PERIOD (TEN PLANTS IN EACH TREATMENT)

FOLLOWING PHOTOPERIODS AS INDICATED, ALL LOTS SUBJECTED TO 12-HOUR DARK PERIOD AND THEN TO LONG DAY IN GREENHOUSE	FLOWERING CONDITION* AFTER 3 WEEKS UNDER LONG DAY IN GREENHOUSE
2 hours of bright sunlight	5 infl. pr., 3 fl., pr., 2 veg.
4 hours of bright sunlight	10 fl. pr.
6 hours of bright sunlight	10 fl. pr.
8 hours of bright sunlight	5 fl. pr., 5 mac. fl. & fr.
10 hours of bright sunlight	4 fl. pr., 6 mac. fl. & fr.
10 hours Mazda light at 10 foot-candles	all veg.
10 hours Mazda light at 100 foot-candles	7 fl. pr., 3 infl. pr.
Controls	10 veg.

* See footnote, table 5.

tionship which seems somewhat similar to the one found in soybean. A long dark period (12 hours) may or may not result in photoperiodic induction, depending upon the duration and intensity of the previous photoperiod. Plants which have been exposed to twelve cycles, each consisting of 3 minutes of light and 3 hours of darkness, are not photoperiodically induced when exposed to a long dark period of 12 hours. They are not induced if the 12-hour dark period is preceded by exposure to illumination of 10 foot-candles from a Mazda lamp for 10 hours, but they are induced if the illumination is 100 foot-candles for this same period. Thus the long dark period must be preceded by a photoperiod of more than 10 foot-candles' intensity if induction is to result. Not only is the intensity but also the duration of the light important during this photoperiod which precedes the

long dark period. An intensity of 100 foot-candles for 10 hours was more effective than bright sunlight for 2 hours, but not nearly so effective as bright sunlight for 10 hours.

While these experiments indicated that the photoperiod which precedes the long dark period plays an important part in determining whether or not exposure to a long dark period will result in initiation of floral primordia, it still remained to be determined just what part, if any, was played by exposure to light just subsequent to the dark period. In both the preceding experiments the long dark period was terminated by exposure to a long photoperiod of bright light (1200 foot-candles or more), since the plants were removed from the dark-room and immediately placed under high intensity of illumination from an arc light or in bright sunlight in the greenhouse. In another series of experiments plants were exposed to a photoperiod in the greenhouse, to a 12-hour dark period, and then immediately to varying types of photoperiod.

EXPERIMENT 9.—One hundred and thirty pots of plants were removed from the greenhouse at 5:00 P.M. and placed in the dark-room for 12 hours. Subsequent to this long dark period, during which photoperiodic induction presumably took place, the plants were divided into various lots, some lots subsequently receiving continuous illumination, some continuing in complete darkness, some receiving photoperiods of low intensity, and so on (table 7; fig. 5).

Floral initiation took place on some plants of each lot, following exposure to one 12-hour dark period, whether the plants were subsequently placed under continuous light or continuous darkness, although only a few plants initiated floral primordia during a period of 2 weeks of total darkness while every plant initiated floral primordia if returned to continuous photoperiod of high intensity in the greenhouse. Of the plants placed on continuous light of 40 foot-candles subsequent to the induction period, three out of twelve remained vegetative, and of the nine that showed floral initiation five were still in the "inflorescence primordia" stage after 3 weeks. Thus high light intensity, following the induction period, stimulates the initiation and development of flowers. The fact that plants may initiate floral primordia while in darkness indicates that, for induction, the photoperiod which ordinarily follows such an induction period apparently

is not always absolutely necessary. The results do show, however, that exposure to light, even though it is only of 40 foot-candles' intensity, does exhibit some stimulative effect upon the development of floral primordia after a long dark period of a photoinductive cycle.

TABLE 7

EFFECT OF PHOTOPERIODIC TREATMENT SUBSEQUENT TO SINGLE INDUCTION PERIOD OF 12 HOURS OF DARKNESS ON FLORAL INITIATION IN XANTHIUM

PLANTS REMOVED FROM GREENHOUSE AT 5 P.M. AND EXPOSED TO 12-HOUR DARK PERIOD, IMMEDIATELY FOLLOWED BY TREATMENTS INDICATED	NO. OF PLANTS PER TREATMENT	FLOWERING CONDITION* AT END OF TREATMENT
Continuous light in greenhouse for 3 weeks	10	10 fl. pr.
Continuous darkness for 2½ weeks	16	5 infl. pr., 11 veg. or dead
Long photoperiod in greenhouse for 3 weeks	10	10 fl. pr.
Continuous light of 40 foot-candles for 3 weeks	12	4 fl. pr., 5 infl. pr., 3 veg.
16 hours arc light, then 3 weeks at 40 foot-candles	8	8 fl. pr.
PLANTS PLACED ON LONG PHOTOPERIOD IN GREENHOUSE FOR 3 WEEKS SUBSEQUENT TO TREATMENTS INDICATED		
Five cycles, each consisting of 12-hour photoperiods and 12 hours of darkness	11	11 mac. fl. & fr.
24-hour photoperiod of 40 foot-candles	10	4 fl. pr., 4 infl. pr., 2 veg.
3 minutes of 5000 foot-candles, 6 hours dark, then 24 hours of 1200 foot-candles	12	10 fl. pr., 2 infl. pr.
30 minutes of 1200 foot-candles, 6 hours dark, then 24 hours of 1200 foot-candles	11	10 fl. pr., 1 infl. pr.
4 hours of 1200 foot-candles, 6 hours dark, then 24 hours of 1200 foot-candles	10	8 fl. pr., 2 infl. pr.
8 hours of 1200 foot-candles, 6 hours dark, then 24 hours of 1200 foot-candles	10	10 fl. pr.
16 hours of 1200 foot-candles, 6 hours dark, then 24 hours of 1200 foot-candles	10	10 fl. pr.

* See footnote, table 5.

The foregoing experiments did not indicate the effect which light conditions following induction may have upon the continued development of floral primordia. A number of the plants exposed to continuous darkness died, the rest were very weak before completion of the experiment, and all plants at 40 foot-candles made poor growth and were yellowish in appearance while those in the greenhouse were green and thrifty. Other experiments, however (fig. 5: VII, VIII), demonstrated that it is the light condition prevailing during the first

16–24 hours following induction which largely determines the rate of development of the floral primordia. Plants which after induction were exposed to 24 hours of low light intensity (40 foot-candles) and

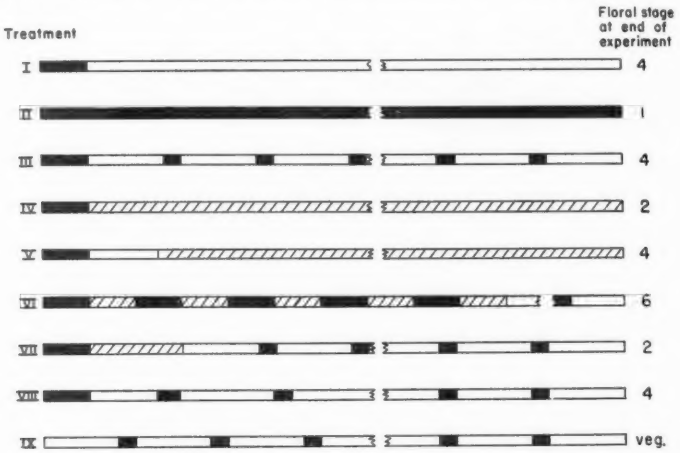

FIG. 5.—Effect of various photoperiodic treatments on *Xanthium* subsequent to photoinductive cycle. Solid black indicates periods of darkness; white, periods of light; barred sections, periods of reduced light intensity. The 12-hour phase of inductive cycle is indicated at the left for each treatment. Designation of floral stage at end of experiment is arbitrary and gives a convenient measure of relative rates of development (cf. table 7). All plants except controls (IX) were removed from the greenhouse at 5:00 P.M. after a bright warm day and exposed to 12 hours of darkness. Subsequently: I, received continuous light in greenhouse for 3 weeks; II, continuous darkness for 2½ weeks; III, cycles of 18–20 hours of light and 6–4 hours of darkness in greenhouse for 3 weeks; IV, continuous illumination at 40 foot-candles for 3 weeks; V, 16 hours illumination at 1200 foot-candles, then at 40 foot-candles for 3 weeks; VI, four and one-half cycles, each consisting of 12 hours of illumination at 40 foot-candles and 12 hours of darkness, and then 3 weeks in greenhouse on cycles with 18–20 hours of light and 6–4 hours of darkness; VII, 24-hour photoperiod at 40 foot-candles, and then 3 weeks in greenhouse as in VI; VIII, 16-hour photoperiod of 1200 foot-candles, 6 hours of darkness, 24 hours of 1200 foot-candles, and then 3 weeks in greenhouse as in VI; IX, controls, received no induction period but remained for 3 weeks under conditions of long photoperiod.

then placed on long photoperiod in the greenhouse did not develop flowers any more rapidly than did plants which received low intensity continuously. The former plants at the time of dissection were more thrifty in appearance and seemed to have abundant food

reserves, in contrast to the weak growth made by the latter. On the other hand, plants which were exposed to 16 hours of illumination from the arc light (1200 foot-candles) and then transferred to continuous low intensity (fig. 5: V) developed flowers as rapidly as did those plants which immediately after induction were placed under continuous light of high intensity (fig. 5: I). Evidently a low intensity of light following the 12-hour dark phase of the induction period does not stimulate the rate of floral development to so great an extent as does a high intensity, but it does stimulate more than does darkness. In the cases of treatment with several cycles, each consisting of 12 hours of darkness and 12 hours of low light intensity, the stimulation of floral development may be very great (fig. 5: VI).

Because of these results, and the facts that an exposure to 16 hours of bright light following the dark phase of an induction period seemed to stimulate the rate of floral development and a 6-hour dark period following such periods of bright light had little or no effect upon the rate of floral bud development (cf. fig. 5: V and VIII), experiments were devised in which the lengths of the periods of bright light were varied but each was followed immediately with a 6-hour dark period.

EXPERIMENT 10.—Two hundred vegetative plants of *Xanthium* were placed in the darkroom at 5:00 P.M. after a warm bright day in the greenhouse and exposed to 12 hours of darkness. Subsequent to this long dark period the plants were divided into ten lots of twenty plants each. One lot was continued in the darkroom for 6 more hours while another lot was placed in the room illuminated by the arc light for 12 hours and thence to conditions of long photoperiod in the greenhouse. The remaining eight lots were transferred to a room illuminated by an arc light. Here the respective lots remained for the following periods: some for 3, 10, or 30 minutes; others for 1, 2, 4, 6, or 8 hours. Subsequent to their respective exposure to bright light, all lots were transferred to a darkroom for 6 hours. At the end of this dark period they were returned to the room illuminated by the arc for at least 12 hours. When the day had become bright the following morning, all were removed to the greenhouse. After 3 weeks of the usual exposure to long photoperiod the plants were carefully dis-

sected. The various stages of development were arbitrarily classified into five groups, ranging from vegetative through four stages of inflorescence and floral development. The average stage of development was determined for each group (table 8). These results are inconclusive but indicate that a short photoperiod following the 12-hour dark phase of a photoinductive cycle of induction results in a

TABLE 8

EFFECT OF VARIOUS SHORT PHOTOPERIODS IMMEDIATELY FOLLOWING 12-HOUR DARK PHASE OF A PHOTOINDUCTIVE CYCLE ON FLORAL INITIATION IN XANTHIUM

VEGETATIVE PLANTS REMOVED FROM GREENHOUSE AT 5:00 P.M. AND EXPOSED TO 12-HOUR DARK PERIOD, THEN TO VARIOUS CYCLES INDICATED. SUBSEQUENTLY PLACED IN ROOM ILLUMINATED BY ARC LIGHT FOR AT LEAST 12 HOURS, REMAINING THERE UNTIL THEY COULD BE TRANSFERRED TO BRIGHT SUNLIGHT IN THE GREENHOUSE THE NEXT DAY

LENGTH OF PHOTOPERIOD (1200 FOOT-CANDLES)	LENGTH OF DARK PERIOD (HOURS)	NO. OF PLANTS IN EACH TREATMENT	NO. OF PLANTS WITH FLORAL PRIMORDIA	AVERAGE STAGE OF FLORAL DEVELOPMENT*
0 minutes	6	20	20	2.0
3 minutes	6	19	18	2.1
10 minutes	6	20	19	2.6
30 minutes	6	20	17	1.9
1 hour	6	20	20	2.6
2 hours	6	20	20	2.8
4 hours	6	19	19	2.8
6 hours	6	20	17	2.1
8 hours	6	20	19	2.8
12 hours	0	20	20	3.2

* The various stages of development exhibited were arbitrarily classified into five groups: vegetative and four stages in inflorescence and flower development.

slower rate of development than a long one. They confirm the results recorded in table 7, that dark periods of 6 hours or less after the photoinductive cycle have no effect on floral initiation or development.

SO-CALLED LONG-DAY PLANTS, DILL AND BEET

As has been shown, *Xanthium* and Biloxi soybean initiate floral primordia only when exposed to photoinductive cycles made up of both light and dark periods. Dill (*Anethum graveolens* Linn.), as grown under the varying cycles of light and dark periods occurring in nature, would be classified as a long-day plant (**6**). Among many other experiments not cited here, however, one was carried out during

early summer in which seeds of dill were planted in pots in soil so near the surface that most of them actually were visible when viewed from above. The pots were immediately placed in the greenhouse where they received natural illumination during the day and artificial illumination of about 100 foot-candles throughout the night. The seeds, seedlings, and developing plants were thus exposed to continuous light. Within 5 weeks most of the plants were in flower, although small and weak with slender flower stalks and relatively few foliage leaves. It is obvious that this plant requires no dark period whatsoever during growth and development to differentiate floral primordia and blossom.

The beet (*Beta vulgaria*) as ordinarily grown is a biennial, flowering during the second season after planting the seeds. Seeds of an annual strain were obtained from Dr. Ewbanks Carsner, who designated it, in so far as its flowering was concerned, as a long-day plant. Many experiments have been conducted with it in the greenhouse. The following results were obtained from some experiments during spring and summer and are pertinent here.

Seeds were planted in ordinary greenhouse flats in January. These were placed on trucks in the greenhouse and exposed to cycles of 9 hours of natural daylight and 15 hours of darkness. The temperature varied during this work but was never below 60° F. After the seedlings had developed four or five foliage leaves and were of sufficient size they were transplanted singly to $3\frac{1}{2}$-inch clay pots. The plants continued on this same cycle until used in the experiment, at which time they were about 6 weeks old. Only uniform plants were used in the several experiments.

In late March ninety-six uniform plants were transferred to cycles, each consisting of approximately 19 hours of light and 5 hours of darkness. Subsequently, every few days eight plants were returned to conditions in which the cycle consisted of 9 hours of light and 15 hours of darkness. This was done in order to determine the number of long photoperiods necessary for induction. The results indicated that more than fifteen long photoperiods were required, although the exact number was not determined with accuracy at this time. Later experimentation showed that the induction period lay somewhere between fifteen and twenty cycles, consisting of 19 hours of light and 5 hours of darkness. These experiments are not dis-

cussed in detail although the results from one of them, carried out at the same time as the experiments listed below, are included in the table with them for comparison.

On May 11, 620 vegetative plants growing on cycles of 9-hour light and 15-hour dark periods were selected for uniformity. Ten were continued on this cycle as controls. These remained vegetative. The remaining 610 were all exposed to ten cycles, each consisting of 18- to 20-hour light periods and 6- to 4-hour dark periods. Subsequent to this 10-day period, 600 of the plants were returned to cycles of short photoperiod, as noted above, and the remaining ten were continued on the cycles of long photoperiod. Subsequent treatment of the plants and the results obtained are given in table 9. The results were recorded 4 weeks after the last transfer was made. The date of harvesting was sufficiently late so that presumably any plants responding to the treatment would have had time to flower.

These results indicate that this particular strain of beet requires from fifteen to twenty cycles of long photoperiods for induction, whether such photoperiods are received in direct succession or received as two separate treatments with varying numbers of cycles of short photoperiod or long dark period intervening. This seems to be true even though as many as sixteen cycles of short photoperiod intervene between the two treatments with cycles of long photoperiod. When as many as thirty-two cycles of short photoperiod intervene, there are indications that a few additional cycles of long photoperiod may be necessary in order to complete the induction; but it is certain that a treatment with as many as thirty-two cycles of short photoperiod will not nullify to any appreciable extent the effect of a previous treatment with cycles of long photoperiod, even though the treatment with the latter was not sufficiently prolonged to bring about the induction of flowering or any visible change in the growth condition of the plant.

Discussion

Beet and dill, the two long-day plants studied, differ in many respects from the two short-day plants, *Xanthium* and Biloxi soybean, so far as their flowering responses are concerned. Dill may be germinated and grown in continuous light and within a few weeks

TABLE 9

FLORAL RESPONSES OF BEET PLANTS SUBJECTED TO VARIOUS NUMBERS OF PHOTOPERIODIC TREATMENT

TYPES OF PHOTOPERIOD TREATMENT	TOTAL NO. OF CYCLES OF LONG PHOTOPERIOD TO WHICH PLANTS WERE EXPOSED, TREATMENT IN MANY CASES BEING INTERRUPTED BY CYCLES OF SHORT PHOTOPERIOD AS INDICATED IN COLUMN 1											
	14	16	18	20	22	24	26	28	30	35	40	45
A. Controls: all cycles of long photoperiod given in consecutive order	7 veg., 3 fl.	1 veg., 8 fl.	1 veg., 8 fl.	1 veg., 8 fl.								
B. Two cycles of short photoperiod between exposure to 10th and 11th cycles of long photoperiod.	10 veg.	7 veg., 3 fl.	2 veg., 8 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.
C. Four cycles of short photoperiod between exposure to 10th and 11th cycle of long photoperiod	10 veg.	9 veg., 1 fl.	5 veg., 5 fl.	7 fl., 3 veg.	10 fl.	9 fl., 1 dead	10 fl.	10 fl.	9 fl., 1 dead	10 fl.	10 fl.	10 fl.
D. Eight cycles of short photoperiod between exposure to 10th and 11th cycles of long photoperiod	10 veg.	7 veg., 3 fl.	2 veg., 8 fl.	10 fl.	9 fl., 1 dead	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.
E. Sixteen cycles of short photoperiod between exposure to 10th and 11th cycles of long photoperiod	10 veg.	5 veg., 5 fl.	2 veg., 8 fl.	10 fl.	9 fl., 1 dead	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.
F. Thirty-two cycles of short photoperiod between exposure to 10th and 11th cycles of long photoperiod	10 veg.	10 veg.	9 veg., 1 fl.	7 veg., 3 fl.	3 veg., 7 fl.	1 veg., 9 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.	10 fl.

develop to the flowering stage with no dark period whatsoever, a fact which is not true for either *Xanthium* or the soybean. Thus it appears that, whatever effect exposure to a dark period may have in the latter two plants, darkness is not required for photoperiodic induction in dill. It is not yet clear whether the particular variety of beet used could be induced to flower without exposure to darkness, since such experiments have not been conducted. It is clear that beet differs from Biloxi soybean and *Xanthium* in that photoperiodic induction occurs when exposed to cycles having relatively long photoperiods (18–20 hours) and short dark periods. There is also a marked cumulative effect of exposure to such cycles even though the cycles may be interrupted by those which are not favorable for induction.

Biloxi soybean and *Xanthium* appear to be similar in that, for photoperiodic induction to take place, they must be exposed to cycles of light and darkness in which the light periods are of a certain intensity and duration and the dark periods of a definitely minimum duration. Since most of the experiments recorded in this paper have dealt with Biloxi soybean and *Xanthium*, and since the few responses of beet and dill studied seem to differ in many respects from them, this discussion deals entirely with an attempt to analyze the responses of Biloxi soybean and *Xanthium*.

Since the specific length and character of both the photoperiod and the dark period determine the results of photoperiodic induction in both plants, it appears that determinative reactions take place during both phases of the cycle, and also that there is an interaction among them. For convenience and brevity of reference, the changes or conditions which arise owing to exposure to light may be designated as A, those owing to darkness as B, and the possible summation or resultant changes related to A and B may be referred to as C. Thus A, B→C.

It is assumed that through the medium of C the observable effects, such as differentiation of floral primordia, floral development, and the like are manifested. Through C, therefore, both A and B would at present have to be investigated. Experimental evidence indicates this is possible. Thus manipulations of conditions during the light period which affect the expressions of C have been

made, and are of such character that it is clear they must be related specifically only to conditions prevailing during the light period. The same is true for other experiments related to the conditions during the dark period. During periods of darkness the degree of accumulated change in A might remain the same or gradually disappear; and similarly the degree of change in B might accumulate during periods of darkness and either persist or disappear during periods of light. The effectiveness of A and B and the relative persistence of either or both during particular conditions of cyclic alternation of light and darkness might presumably determine the changes relating to and the effectiveness of C. As in the cases of A and B, C could also disappear, dependent upon the various environmental factors involved. The experiments listed in this paper were largely designed to investigate these various possibilities and are discussed in relation to them.

Evidence relative to A and factors influencing it

Biloxi soybean.—Many suggestions have been made in attempts to explain various types of photoperiodic evidence. Some have considered the light phase as primarily important, others the dark phase, and still others various interactions of the two. Borthwick and Parker (2) have shown that Biloxi soybean will initiate floral primordia as the result of exposure to three 24-hour cycles, each consisting of a short light period and a long dark period, but will not initiate floral primordia as the result of exposure to 72 hours of darkness nor as the result of exposure to continuous light. As has been shown in experiments 1, 2, and 3 and illustrated by figure 2, under the influence of cycles in which each dark period is of 16 hours' length the number of floral primordia initiated is dependent upon the duration of the light period, increasing up to exposures of 11 hours and decreasing with longer exposures. Also the number of primordia formed is dependent upon the intensity of the illumination, increasing with increasing intensities (experiment 6 and fig. 4).

Xanthium.—Previous work (5) has shown that floral initiation in *Xanthium* is ordinarily limited by reactions taking place during the dark period. The plants under 24-hour cycles of alternating light and dark periods, in which the light period exceeds 4 hours, pre-

sumably experience an increased effectiveness of A and initiate floral primordia whenever the plant is exposed to cycles having dark periods of 9 hours or more, which permits an increased effectiveness of B. In order to demonstrate the effect of conditions which influence A, it was first necessary to obtain plants at a minimum of A. The procedure actually used to secure such plants is outlined in experiments 7 and 8. After being removed from the greenhouse, the plants were not immediately exposed to a long dark period, but instead to short cycles each consisting of 3 minutes of light and 3 hours of darkness. After exposure to four such cycles (experiment 7 and table 5) the plants did not become photoperiodically induced, even though subsequently exposed to a long dark period. The possible explanation of such responses might be as follows. During such short cycles, the opportunity for an increase in the effectiveness of A is slight since the duration of each photoperiod is only 3 minutes. Not only does A not increase greatly under these conditions but it actually decreases until, after four cycles, it becomes so slight that photoperiodic induction does not occur. During exposure to such short cycles B does not increase in effectiveness because the dark periods of each cycle are too short, but there was abundant opportunity for increase of B during the 12 hours of darkness following the last cycle. The progression A, B→C did not follow because of the deficiency of A. That the deficiency of A limited the behavior is further indicated as probable by experiment 8. Plants were exposed to twelve of the short cycles. Such plants failed to initiate floral primordia if they were immediately exposed to 12 hours of darkness, but they did initiate floral primordia if, just previous to exposure to the 12-hour dark period, they were exposed to a photoperiod of 2 hours of bright sunlight or 10 hours of light from a Mazda lamp at 100 foot-candles so that there was an opportunity for an increased effectiveness of A. Under such conditions the rate of floral development was roughly proportional to the duration and intensity of the light.

Thus in *Xanthium*, as in Biloxi soybean, it appears that there are definite, modifiable conditions during exposure to light which quantitatively influence the results of the photoperiodic reaction as a whole.

Evidence relative to B and factors influencing it

Xanthium.—Repeated experiments with *Xanthium* (**5, 7**) have shown that photoperiodic induction does not take place unless the dark period of any cycle exceeds $8\frac{1}{2}$ hours. Whatever the reactions are that take place during exposure to darkness, they seem to be inhibited by light (**5, 11**) even of very low intensity. A dark period of 9 hours which would be effective in floral initiation may be completely ineffective if interrupted by as little as one minute of light. Exposure of plants to low temperatures during the dark periods greatly reduces their effectiveness, a much longer time being required under such conditions for induction to take place (**5, 3**). These results, together with the results of experiment 7, indicate that there is an increasing effectiveness of B during exposure to darkness, and that, whatever B is, there is no response resulting in C up to a certain threshold value. At temperatures of 65°–80° F., this threshold value is passed after $8\frac{1}{2}$–9 hours of darkness.

The results also indicate that, up to the threshold value, whatever degree of B has been attained during exposure to darkness, it may be completely or almost completely negated by a very brief exposure to light. As already stated, one minute of light which interrupts a 9-hour dark period completely nullifies the effect of the entire period of darkness prior to the interruption, and another period of complete darkness (9 to 12 hours) is necessary to be effective (experiment 7).

Biloxi soybean.—That photoperiodic induction in Biloxi soybean will not take place unless the dark period is definitely more than a certain minimum duration is strikingly illustrated in figure 1 and experiments 2 and 3. Borthwick and Parker have obtained with Biloxi soybean much the same results as were obtained with *Xanthium* with brief exposures to light (**2**) and with exposures to low temperatures during the dark period (**3**).

A consideration of the two plants, in so far as conditions of A and B are concerned, demonstrates their many similarities. As already suggested, it would seem likely that in both plants the A, B→C relationship might hold. The effectiveness of A accumulates during exposure to light and slowly decreases during exposure to darkness. In conjunction with B which increases during exposure to darkness

and may decrease very rapidly during exposure to light, C results, and floral initiation takes place. Many experiments show that the rate of development of flowers, fruits, and the like is influenced by environmental factors, especially the level of nutrition (**4, 9, 10**) subsequent to the photoinductive period.

Evidence relative to C and factors influencing it

Xanthium.—The direct results, such as floral initiation, which are assumed to be specifically related to C, also show that it probably varies quantitatively and may be influenced by conditions immediately following the photoinductive period. Thus in *Xanthium* initiation of floral primordia may follow a single 24-hour cycle of 12 hours of light and 12 hours of darkness. This is true whether the plants are maintained immediately subsequently in total darkness or in continuous light (experiment 9 and fig. 5). Also, plants which have been induced may continue to flower and set fruit even though subsequently exposed for 7 months to continuous light (**5, 10**).

The responses to C do not seem to represent strictly an "all or none" reaction. It has been found repeatedly that while one cycle of treatment may result in floral initiation, two cycles result in more rapid development of the primordia into flowers and fruit, three cycles in still more rapid development, and so on. It seems probable therefore that the effectiveness of C is not rapidly destroyed and may be intensified, resulting in a more rapid development of primordia into flowers and fruits, depending upon nutritional and other environmental factors as well. The experiment recorded in table 7 and figure 5 could be interpreted on this basis.

Biloxi soybean.—The evidence in the case of Biloxi soybean relating to C is of much the same nature as in *Xanthium*, in that induction takes place only in relation to a certain number and character of photoinductive cycles (experiments 1, 2, 3, 4, and 5; figs. 1, 2, 3, and 4). While in *Xanthium* the number of photoinductive cycles to which plants were exposed affected the rate of floral development, in Biloxi soybean not only does the rate of floral development depend upon the number of cycles of treatment (**1**), but also the total number of floral primordia formed increases almost in direct proportion

to the number of photoinductive cycles of treatment. One cycle does not result in the initiation of any primordia (table 2; fig. 3).

In Biloxi soybean, in contrast to *Xanthium*, while C may arise in much the same manner, several photoinductive cycles are required for it to become sufficiently effective to result in the initiation of floral primordia. Evidence for such a hypothesis is available from at least two sources. First, the evidence from the present experiments as well as those of others indicates that C may be intensified through two or three consecutive photoinductive cycles before there is initiation of floral primordia. Second, the experiments of LONG (7) showed that, following three photoinductive cycles, floral initiation took place to a certain extent and then when the plants were placed under conditions of long photoperiod no more flower primordia were initiated. If another series of photoinductive cycles of short photoperiod were used on the same plants, however, then additional floral primordia would be differentiated by them, the number of primordia being in proportion to the number of such cycles employed. Thus it was possible to induce plants to flower, have them develop vegetatively for a time, and then develop primordia more or less at will; but in each instance the plants behaved as though they had not been previously induced, or as though there was no prolonged carryover of the effects of C unless the photoinductive cycles were virtually consecutive.

From these experiments it could be assumed that C increases in soybean much as it does in *Xanthium*, as the result of exposure to photoinductive cycles. Differing from *Xanthium*, however, C is insufficient in any one cycle to bring about subsequent floral initiation, or it may not persist but disappear rather rapidly during exposure to light. Such an assumption could explain the results of LONG. Unless exposure to the photoinductive cycles occurred in direct succession, there was no effectiveness of C from one cycle to another, owing to its disappearance over intervening periods, and it never passed the threshold value. In order that additional primordia might be initiated, it was necessary to subject plants to several photoinductive cycles.

Such an explanation also would cover the results illustrated in

figure 2. Cycles which include photoperiods in excess of 20 hours do not result in photoperiodic induction even though the dark periods of such cycles are 16 hours in duration. Since A increases in effectiveness during light, presumably a 20-hour photoperiod should result in an intensification of A; and since a 16-hour dark period is long enough for B to increase above its threshold value, then in a cycle consisting of a 20-hour photoperiod and a 16-hour dark period a certain effectiveness of C should result. An explanation for the failure of such cycles to result in floral initiation could be that during the long photoperiod of each cycle the conditions of C which resulted during the previous cycle disappeared. Thus, in figure 2, the downward slope of the curve of floral initiation with increasing photoperiod could represent the effect of photoperiod on the dissipation of C.

Summary

1. Experimental work has been presented on the effect of various cycles of light and darkness on photoperiodic induction of Biloxi soybean, *Xanthium*, dill, and beet.

2. Experimental evidence has indicated that in both the soybean and *Xanthium* photoperiodic induction is dependent in part upon responses which occur as the result of exposure to light. For purposes of discussion, these responses have been referred to as A.

3. Experimental evidence has indicated that in both *Xanthium* and soybean photoperiodic induction is dependent in part also upon responses which result from exposure to darkness. For purposes of discussion, these have been referred to as B. Photoperiodic induction results only after periods of darkness which exceed a certain minimum length.

4. For convenience and brevity of reference, the changes or conditions related to exposure to light may be designated as A, those owing to darkness as B, and the resultant changes related to A and B may be referred to as C.

5. The establishment of the categories A, B→C as here suggested is an aid toward an interpretation of the behavior of *Xanthium* and Biloxi soybean. Its extension to the interpretation of the photoperiodic behavior of other plants may be possible.

6. Additional experiments, so performed that they may be subjected to such a type of analysis, are in progress.

The assistance given by A. W. NAYLOR, L. K. MANN, and W. E. SNYDER in carrying out some of these experiments is greatly appreciated.

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EFFECT OF NAPHTHALENE ACETIC ACID AND NAPHTHALENE ACETAMIDE ON NITROGENOUS AND CARBOHYDRATE CONSTITUENTS OF BEAN PLANTS

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(WITH TWO FIGURES)

Introduction

The application of alpha naphthalene acetamide to the stems of young bean plants results in such characteristic histological and morphological responses as inhibited longitudinal growth of internodes without marked increase in stem thickness, and increased cambial activity in the stem near the treated region, with formation of a relatively large amount of secondary thickening (**2, 4**). Comparable application of alpha naphthalene acetic acid results in meristematic activity of cells of several tissues of the stems near the treated region, and finally in the initiation of numerous root primordia on the stem (**1, 4**). Elongation of the internodes and expansion of the leaves of the terminal buds is retarded as a result of application of naphthalene acetamide, and also naphthalene acetic acid, to the stems of bean plants. Similar responses have been observed in plants sprayed with lanolin emulsions containing these compounds (**4**).

In the present investigation some chemical responses that resulted from treating plants with naphthalene acetamide are compared with those that resulted when the plants were treated with naphthalene acetic acid or left untreated.

Lanolin mixtures of naphthalene acetamide and naphthalene acetic acid were applied individually to the stems of the plants. These were later analyzed and the amount, and to some extent the type, of carbohydrate and nitrogenous compounds present in several portions of the plants compared.

Methods

Approximately 3000 kidney bean plants were grown in sand with a complete nutrient solution containing nitrogen in the form of calcium

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nitrate. The nutrient was first applied when the plants emerged from the sand; sufficient later applications were made to keep the sand at optimum moisture content. The greenhouse temperature varied between 70°–75° at night and 75°–85° F. during the daytime. The light intensity was relatively high, as clear weather prevailed during the experiment.

Two per cent lanolin mixtures of alpha naphthalene acetamide and alpha naphthalene acetic acid were applied individually as a ring 2 mm. wide around the first internodes, midway between the first and second nodes, when the plants were 10–12 cm. tall and the first internodes approximately 15 mm. long. Control plants were treated in a similar way, using pure lanolin. The treatments were distributed evenly throughout the entire number of plants grown. The plants were harvested 12 days following treatment, at which time they showed marked responses and had not yet produced flowers.

Plants were carefully removed from the sand and the roots washed and dried on paper toweling. Approximately 500 plants from each treatment were used for nitrogen determinations. These were divided into the following portions: (1) roots and hypocotyls; (2) first internodes; and (3) tops, including all leaves, internodes, and petioles above the first internode.

Samples for carbohydrate determinations were collected from 300–500 plants in each treatment and divided into five portions: roots, hypocotyls, first internodes, primary leaves, and all parts above the first internode other than primary leaves (which were designated tops).

Fresh tissue analyzed for nitrogenous compounds was ground to a fine pulp and moisture samples were taken, the solids from which were later used for total nitrogen determinations by means of the Kjeldahl method. Soluble nitrogen fractions were removed from aliquots repeatedly ground with cold water in quartz sand and filtered through cloth. Following clarification with acetic acid, ammonia and nitrate nitrogen in the extract were determined as described by SESSIONS and SHIVE (**7**), and the total nitrogen by the Kjeldahl method.

Sugars were extracted with 80 per cent alcohol from tissue pre-

viously dried and ground to 60 mesh. The extracts were cleared with neutral lead acetate and the non-reducing sugar hydrolyzed with invertase. Reducing power of the resulting solutions was determined by a micromethod (**5**) with modifications as suggested by PHILLIPS (**6**), the copper being dissolved in ferric alum and the iron titrated with potassium permanganate.

Starch was digested with saliva in samples ground to 100 mesh, hydrolysis was completed as described by LOOMIS and SHULL (**3**), and reducing power of the cleared solutions was determined by the method described.

Experimental results

GROWTH RESPONSES

Two hours after treatment with naphthalene acetic acid the plants showed marked stem curvatures, which persisted throughout the experiment. Tumors developed on the first internodes near the treated region within 3–4 days after treatment. Plants treated with the acetamide mixture or pure lanolin showed no curvatures or tumor formation during the experiment. Both naphthalene acetic acid and naphthalene acetamide inhibited the expansion of internodes, leaves, and petioles. Terminal buds failed to increase appreciably in size during the 12 days following application of naphthalene acetic acid to the first internodes. At the time of harvest, first internodes treated with naphthalene acetamide and naphthalene acetic acid measured approximately 2 inches in length, while those to which pure lanolin was applied were approximately 3 inches.

Numerous roots formed on the hypocotyls and first internodes of plants treated with the acid, and a smaller number were evident only on the lower part of the hypocotyls of plants treated with the acetamide.

CHEMICAL RESPONSES

Naphthalene acetamide and naphthalene acetic acid, applied in lanolin to the first internodes of bean seedlings, resulted in chemical responses in the stems near the treated region, and also in all other portions of the plants that were analyzed. Considering the plant as a whole, the amount of solid matter synthesized by those treated with the acetamide and the acid was less, while the water content meas-

ured as percentage moisture in fresh tissue was greater than that of controls (tables 1, 2). All portions, except the primary leaves of

TABLE 1

WEIGHT OF FRESH AND DRY TISSUE OF BEAN PLANTS 12 DAYS FOLLOWING TREATMENT WITH 2 PER CENT LANOLIN MIXTURE OF NAPHTHALENE ACETAMIDE AND NAPHTHALENE ACETIC ACID, AS COMPARED WITH OTHERS TREATED WITH PURE LANOLIN. FIGURES REPRESENT GRAMS SOLID MATTER PER 100 PARTS

Plant part	Lanolin		Naphthalene acetamide		Naphthalene acetic acid	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Roots	110.8	11.3	131.8	10.7	115.1	8.6
Hypocotyls	90.1	11.1	90.3	9.7	101.2	9.0
First internodes	37.0	5.6	34.0	5.2	153.1	19.2
Primary leaves	304.9	34.2	307.2	33.2	244.7	29.3
Tops*	620.9	68.7	245.0	28.8	8.2	1.0
Entire plant	1163.7	130.9	808.3	87.6	622.3	67.1

* All portion above second node.

TABLE 2

MOISTURE CONTENT OF PLANTS TREATED WITH 2 PER CENT LANOLIN MIXTURES OF NAPHTHALENE ACETAMIDE AND NAPHTHALENE ACETIC ACID AS COMPARED WITH OTHERS TREATED WITH LANOLIN

Plant part	Lanolin		Naphthalene acetamide		Naphthalene acetic acid	
	Per 100 parts (gm.)	Percentage fresh weight	Per 100 parts (gm.)	Percentage fresh weight	Per 100 parts (gm.)	Percentage fresh weight
Roots	99.5	89.8	121.1	91.8	106.5	92.5
Hypocotyls	79.0	87.6	80.6	89.2	92.2	91.1
First internodes	31.4	84.8	28.8	84.7	133.9	87.4
Primary leaves	207.7	68.1	274.0	89.1	215.4	88.0
Tops	552.2	88.9	216.2	88.2	7.2	87.4
Entire plant	969.8	83.3	720.7	89.2	555.2	89.2

plants treated with the acid, contained a lower percentage of carbohydrate in the form of starch, dextrin, and sugar than did controls (table 3). Similarly the roots, hypocotyls, and first internodes of

TABLE 3

AMOUNT OF STARCH, DEXTRIN, AND SUGAR IN PLANTS 12 DAYS FOLLOWING TREATMENT WITH 2 PER CENT MIXTURE OF LANOLIN-NAPHTHALENE ACETAMIDE AND LANOLIN-NAPHTHALENE ACETIC ACID, AS COMPARED WITH OTHERS TREATED WITH LANOLIN

Plant part	Basis of calculations					
	Per 100 parts (mg.)			Percentage solid matter		
	Lanolin	Naphthalene acetamide	Naphthalene acetic acid	Lanolin	Naphthalene acetamide	Naphthalene acetic acid
	Total sugar					
Roots	215	139	112	1.9	1.3	1.3
Hypocotyls	599	233	198	5.4	2.4	2.2
First internodes	196	130	442	3.5	2.5	2.3
Primary leaves	1300	1162	1436	3.8	3.5	4.9
Tops	2473	778	25	3.6	2.7	2.5
Entire plant	4783	2442	2213	3.6	2.8	3.3
	Starch and dextrin					
Roots	0	0	0	0.0	0.0	0.0
Hypocotyls	278	0	0	2.5	0.0	0.0
First internodes	0	0	0	0.0	0.0	0.0
Primary leaves	992	1162	2110	2.9	3.5	7.2
Tops	1992	1238	.....	2.9	4.3	.....
Entire plant	3262	2400	2110	2.5	2.7	3.1
	Starch, dextrin, and sugar					
Roots	215	139	112	1.9	1.3	1.3
Hypocotyls	877	233	198	7.9	2.4	2.2
First internodes	196	130	442	3.5	2.5	2.3
Primary leaves	2292	2324	3546	6.7	7.0	12.1
Tops	4465	2016	25	6.5	7.0	2.5
Entire plant	8045	4842	4323	6.1	5.5	6.4

plants treated with the acetamide contained a lower percentage of these forms of carbohydrates than did comparable parts of controls. As compared with controls, the plants treated with the naphthalene compounds contained a limited supply of carbohydrate in the form of starch, dextrin, and sugar in tissues adjacent to and below the treated region of the stem.

On the other hand, that portion of the plants above the treatment, including primary and expanded trifoliate leaves of plants treated with the acetamide and primary leaves of those treated with the acid, contained a greater percentage of carbohydrate, mainly in reserve forms (starch and dextrin), than did comparable parts of controls. These results indicate that the expanded leaves of the treated plants were photosynthetically active but any appreciable amount of the synthate was not transported readily to other parts of the plants, where a relatively low concentration of sugars prevailed. The presence of a relatively low percentage of carbohydrate (starch, dextrin, and sugar) in the roots, hypocotyls, and first internodes of plants treated with the naphthalene compounds could in part be the result of three responses: (1) inhibited leaf expansion; (2) inhibited transport of carbohydrates from the leaves; and (3) increased growth (meristematic activity and secondary thickening in the hypocotyls and first internodes) which would tend to deplete the plant of these forms of carbohydrate.

Nitrogen was present in relatively large amounts in plants treated with naphthalene acetamide and also in those treated with naphthalene acetic acid, as approximately 5 per cent of the solid matter of plants given these respective treatments was nitrogen, while the solid matter in control plants contained only 2.2 per cent nitrogen (tables 4, 5). Distribution of nitrogen throughout plants treated with the naphthalene compounds was unlike that of controls and apparently associated with specific growth responses. That portion of stems near the region to which naphthalene acetic acid was applied developed tumors which were made up largely of meristematic cells (fig. 1), and this portion of the plant contained nearly one-half of the water-soluble nitrogenous compounds, and more than one-third of the total nitrogen in the entire plant. Comparable portions

Fig. 1.—Transverse median section of tumor resulting from application (narrow ring) of 2 per cent naphthalene acetic acid-lanolin mixture 96 hours after treatment, showing numerous meristematic cells of the tumor and newly developed roots.

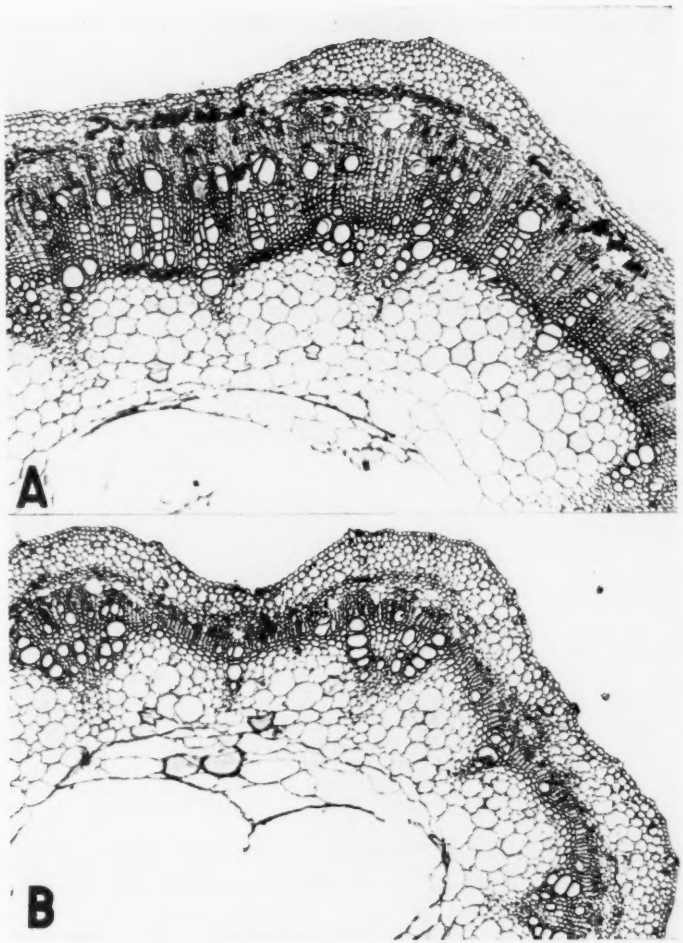

FIG. 2.—*A*, transverse median section through region over which narrow ring of 2 per cent naphthalene acetamide-lanolin mixture had been placed. Section made 216 hours after application. *B*, similar section made from stem treated with lanolin after same period of time. Obvious differences are increased thickening of walls of pericyclic fibers and greater amount of secondary xylem, indicating greater cambial activity in *A*. Cf. fig. 1, same magnification.

of the stems of controls contained less than one-fiftieth of the total nitrogen in the plants.

Tumors did not develop in that portion of stems to which naphthalene acetamide was applied, but instead cambial activity increased and a relatively large number of cells were derived which were characterized by thick walls (fig. 2). A relatively small amount of nitrogen was mobilized in connection with this growth response, as this portion of the stems of plants treated with the acetamide

TABLE 4

AMOUNT OF TOTAL NITROGEN IN PLANTS 12 DAYS AFTER TREATMENT WITH 2 PER CENT MIXTURES OF LANOLIN-NAPHTHALENE ACETAMIDE AND LANOLIN-NAPHTHALENE ACETIC ACID, AS COMPARED WITH OTHERS TREATED WITH LANOLIN

Plant parts	Basis of calculations								
	Per 100 parts (mg.)			Percentage of solid matter			Percentage amount in entire plant		
	Lanolin	Naphthalene acetamide	Naphthalene acetic acid	Lanolin	Naphthalene acetamide	Naphthalene acetic acid	Lanolin	Naphthalene acetamide	Naphthalene acetic acid
Roots and hypocotyls	457.0	567.1	582.6	2.0	2.8	3.3	7.8	13.1	17.3
First internodes	108.6	128.4	1223.0	1.9	2.5	6.4	1.9	3.0	36.2
Leaves	5309.6	3627.0	1572.6	5.2	5.9	5.2	90.4	83.9	46.6
Entire plant	5875.2	4322.5	3378.2	2.2	4.9	5.0	.....	.....	.....

contained no more of the water-soluble nitrogenous compounds and only slightly more total nitrogen than did comparable parts of the stems of control plants.

The hypocotyls and roots of plants treated with the acetamide, and also the acid, contained more nitrogen, particularly in the form of water-soluble nitrogenous compounds, than did comparable portions of controls. The presence of a relatively large amount of nitrogen in the hypocotyls of plants treated with the napthalene compounds was apparently associated with initiation of numerous roots

within the hypocotyls of those treated with the acid, and likewise with initiation of a small number of roots within the hypocotyls of those treated with the acetamide.

TABLE 5

AMOUNT OF WATER-SOLUBLE NITROGENOUS COMPOUNDS AND NITRATE IN PLANTS 12 DAYS FOLLOWING TREATMENT WITH 2 PER CENT MIXTURES OF LANOLIN-NAPHTHALENE ACETAMIDE AND LANOLIN-NAPHTHALENE ACETIC ACID, AS COMPARED WITH OTHERS TREATED WITH LANOLIN

Plant part	Basis of calculations								
	Per 100 parts (mg.)			Percentage total nitrogen			Percentage amount in entire plant		
	Lanolin	Naphthalene acetamide	Naphthalene acetic acid	Lanolin	Naphthalene acetamide	Naphthalene acetic acid	Lanolin	Naphthalene acetamide	Naphthalene acetic acid
	Water-soluble nitrogen								
Roots and hypocotyls	219.5	320.3	345.0	48.0	56.5	59.2	12.8	20.5	22.8
First internodes	56.6	49.9	693.1	52.1	38.9	56.7	3.3	3.2	45.8
Tops*	1440.6	1190.4	475.7	27.1	32.8	30.2	83.9	76.3	31.4
Entire plant	1716.7	1560.6	1513.8	29.2	36.1	44.8	.....	.....	.....
	Nitrate nitrogen								
Roots and hypocotyls	87.4	144.8	139.0	19.1	25.5	23.9	17.9	30.2	41.1
First internodes	20.7	12.0	84.5	19.1	9.3	6.9	4.2	2.5	25.0
Tops	380.7	322.4	115.1	7.2	8.9	7.3	77.9	67.3	34.0
Entire plant	488.8	479.2	338.6	8.3	11.1	10.0	.....	.....	.....

* All portions above first internode.

Application of naphthalene acetic acid to the stems inhibited to a marked degree the extension of internodes and expansion of leaves in the terminal buds during the 12 days following treatment. Comparable applications of the acetamide resulted in similar but less

pronounced effects. The inhibition of growth in the terminal buds of plants treated with the naphthalene compounds was not associated with a limited supply of nitrogen as such in any of the portions of the plants analyzed.

Summary

1. Application of alpha naphthalene acetamide and alpha naphthalene acetic acid to the stems of bean seedlings affected the total amount and distribution of carbohydrate and nitrogenous compounds in various portions of the plants, and also in the plant as a whole. Some of the chemical responses were specifically associated with the application of the acetamide while another type of chemical response resulted when the plants were treated with the acid. In general the chemical responses were closely associated with growth responses, growth by expansion of leaves and extension of internodes being inhibited in that portion of the plants above the treated section of the stem, while meristematic or cambial activity was stimulated in the treated section and hypocotyls.

2. Tumors, made up largely of meristematic cells, developed in that portion of stems to which naphthalene acetic acid was applied. Analysis of this portion of the stems showed them to contain a relatively high percentage of nitrogen, which was present in the form of compounds that were water-soluble. Increased cambial activity and a relatively large amount of secondary thickening resulted in the treated region of stems following the application of naphthalene acetamide. This portion of the stems contained only slightly more nitrogen than comparable sections of stems of control plants, and a relatively small percentage of the nitrogen present was in the form of water-soluble compounds.

3. Plants treated with naphthalene acetic acid, and also those treated with naphthalene acetamide, contained a lower percentage of starch, dextrin, and sugar in the roots, hypocotyls, and first internodes than did controls. The presence of a limited amount of readily available carbohydrate in these parts of treated plants was associated with the development of a relatively small amount of leaf surface. There was also inhibited transport of carbohydrate from the leaves. Growth responses in the stems were characterized by intensive cambial and meristematic activity, and the initiation of roots

within the hypocotyls. Carbohydrate metabolism and transport was so affected as finally to result in a deficiency in the amount of carbohydrate available in those portions of the plants where growth was stimulated for a period following treatment.

4. There was no evidence of nitrogen deficiency, as the percentage of nitrogen present in all portions of plants treated with the acid—and also those treated with the acetamide—was equal to or greater than that in control plants.

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INTERRELATIONS IN THE EFFECTS OF BORON AND INDOLEACETIC ACID ON PLANT GROWTH

FRANK M. EATON

(WITH ONE FIGURE)

Introduction

Some of the symptoms of plants deficient in boron are sufficiently similar to those expected in plants deficient in auxin as to suggest that the role of boron in plant nutrition is closely associated with the formation of auxin and possibly of other plant hormones. The experiments here reported show that indoleacetic acid added to nutrient solutions will partially replace boron.

Plants grown to maturity in sand cultures outdoors have yielded puzzling results in that, with boron concentrations averaging about 0.05 ppm in the nutrient solution, the growth of some fifteen species has been markedly at variance in succeeding summers. In some seasons growth was greatly depressed and there were pronounced deficiency symptoms, whereas in other seasons the same species were normal or nearly so. Analyses of the plant material showed similar concentrations of boron in the normal and deficient plants. Spectroscopic examinations of the plant material for other elements that might have been introduced as impurities replacing boron were negative, as were many culture experiments with elements such as aluminum, gallium, scandium, germanium, and indium, in addition to some of the elements previously tested by BRENCHLEY and WARINGTON (1).

Elongation of the stems, petioles, and roots of plants in minus-boron nutrient solutions is slow or may cease soon after the cotyledons have developed. The rate at which new nodes are formed is affected later. With the tendency for the terminal bud to abort or take on a fasciated appearance, similarly abnormal branches appear in the axils of the leaves. The leaves of cotton and other plants deficient in boron are small, become deeply cupped, and have a peculiarly patched sort of chlorotic mottling. Buckling of the meso-

phyll of boron-deficient leaves indicates a greater depression in the growth of vein structures than of mesophyll. The downward cupping of leaves, as suggested by Dr. H. E. HAYWARD, may reflect also a greater retardation of the growth of the phloem than the xylem elements. Growth of the marginal mesophyll is checked in advance of that adjacent to the main veins, and the formation of lobes of cotton leaves may be partially or wholly repressed. Boron-deficient leaves are usually thickened. Splitting open and corking of veins of boron-deficient plants is sometimes observed. The roots of such plants, as shown in the instance of the pea plant by SOMMER and SOROKIN (**2**), are short and stubby, and the secondary roots make little growth.

The effects of auxin deficiency on plant growth cannot be described very accurately, since conclusions in many instances must be drawn indirectly or by inference. WENT (**4**) states that while there is practically no evidence linking leaf-blade growth with auxin, there is considerable circumstantial evidence pointing to the conclusion that auxin specifically conditions petiole and vein growth. Auxin tends to inhibit the development of lateral branches. Root formation is induced by auxin but other hormones have been found essential to root growth.

Experimentation

In each of the experiments here discussed, Acala cotton plants were grown in Hoagland's solution (5, 5, 2, and 1 millimole per liter respectively of $Ca(NO_3)_2$, KNO_3, $MgSO_4$, and KH_2PO_4) in unaerated quart mason jars. To this solution, which contained less than 0.01 ppm of boron derived as an impurity in the chemicals, was added 0.1 ppm each of zinc and manganese, and sufficient iron citrate. Indoleacetic acid, when used, was added daily and cumulatively.

The first experiment was conducted in the spring, during a period when most of the days were overcast. There were two storms, each of several days' duration. In this experiment, as shown by table 1 and figure 1, the daily addition of 0.01 ppm of indoleacetic acid to minus-boron solutions resulted in greatly increased leaf development. At the time the measurements were made (the 28th day after transferring the seedlings to the culture solutions), 1 ppm of boron had been present in culture A for only 16 days, whereas indoleacetic

acid had been added daily to cultures C, D, and E for 24 days. In other words, boron was added to a parallel untreated culture only after the first response in root growth from indoleacetic acid had been observed. The leaves on plants in culture E were nearly

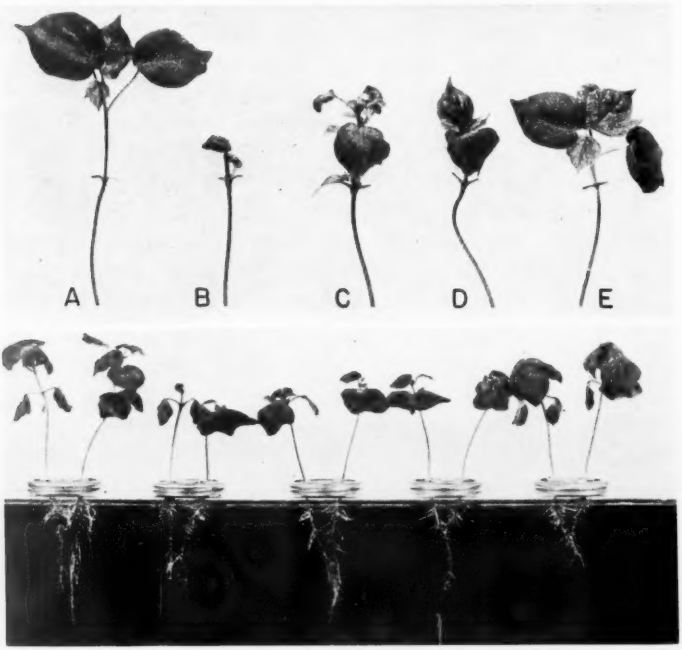

FIG. 1.—Acala cotton plants grown in nutrient solutions. Above, single plant from each culture with cotyledons removed; below, the two remaining plants.

	A	B	C	D	E
Boron added 16 days before photographing, ppm	1	0	0	0	0
Indoleacetic acid added daily for 24 days, ppm	0	0	0.0001	0.001	0.01

normal in appearance, but it is to be noted that they were not supported in the same outward position from the petioles as those of culture A, which received boron. Internode and petiole elongation was promoted by indoleacetic acid, but in the highest concentration the total length of the internodes was only about half as great as in the plus-boron culture. Indoleacetic acid in a concentration of

0.0001 ppm added daily (culture C) induced root development which for a time appeared normal. Later, however, possibly because of accumulation of an unfavorable concentration of indoleacetic acid and because of retarded development of the leaves, the roots developing near the surface of the solution were shorter than in the plus-boron solution. In the higher indoleacetic acid concentrations (cultures D and E) the roots were short.

In a second experiment that included tomatoes and sunflowers as well as cotton, little or no response to indoleacetic acid resulted.

TABLE 1

LEAF DEVELOPMENT OF COTTON PLANTS IN SOLUTIONS WITH BORON AND WITH INDOLEACETIC ACID

	Culture				
	A*	B	C	D	E
Boron, ppm	1	0	0	0	0
Indoleacetic acid added daily, ppm	0	0	0.0001	0.001	0.01
Leaf areas (exclusive of cotyledons) sq. cm.:					
Plant 1	82	4	9	14	82
Plant 2	78	4	10	11	74

*In comparing leaf areas, account must be taken of the fact that boron was added to culture A 16 days before the measurements, whereas cultures C, D, and E had been receiving indoleacetic acid for 24 days.

This test, conducted during a period of bright warm days, was discontinued after about the tenth day.

A third experiment was then set up with cotton plants, using four cultures for each treatment. Half of the cultures were placed in a brightly illuminated greenhouse and half in a muslin-covered lath house. The noonday light intensities in the lath house ranged from 500 to 1000 foot candles and in the greenhouse from 4000 to 7000 foot candles.

The plants in the lath house responded to indoleacetic acid in much the same way as they did in the first experiment. The internode elongation in the higher indoleacetic acid concentrations was somewhat better than in the first test, but it was not so good as in the cultures supplied with boron. Any advantages of indoleacetic acid for root development were uncertain or transitory in this ex-

periment. The plants in the brightly illuminated greenhouse showed little response during the early period of the experiment. All the leaves were small and cupped, and no stimulation of root growth was observed. The greenhouse plants were maintained for a month longer than those in the lath house. At the end of this period each successively higher concentration of indoleacetic acid, including a pair of cultures receiving 0.1 ppm, had produced more and larger leaves than the next lower concentration. The total leaf area of the plants receiving 0.1 ppm indoleacetic acid was possibly eight or ten times as great as the plants in "0-boron 0-indoleacetic acid" solutions, but none of the leaves were normal. The plants receiving boron were in all respects superior to any of the indoleacetic acid plants and had much greater leaf areas.

Because of the polar movement of indoleacetic acid, concentrations in the foregoing experiments that were sufficiently high to induce favorable responses in the growth of leaves and stems were injurious to the roots. Following a suggestion by Dr. E. J. KRAUS, a subsequent test was conducted in which indoleacetic acid was applied to the leaves and stems of cotton plants in solution with 1 per cent each of lanolin and sodium oleate as a spray three times per week. The concentrations used in this test were probably too low, since a substantial increase in the growth of stems and leaves resulted only from the highest concentration, which was 10 ppm.

Vitamin B_1 and yeast extracts were added, in other experiments, to minus-boron solutions alone and in conjunction with indoleacetic acid. These substances did not improve growth nor lessen the severity of boron deficiency symptoms.

Discussion

These experiments provide evidence that boron as an element essential to the growth of plants can in some measure be replaced by indoleacetic acid. In no test, however, were the results obtained with indoleacetic acid equal to the responses that followed additions of boron to nutrient solutions. The findings point to the conclusion that at least one of the functions of boron in plant nutrition is intimately related to the formation of plant hormones. Any interpretation of the results must take into account the experimental

difficulties associated with the movement of indoleacetic acid into the plant and to the active tissues in concentrations neither too high nor too low. Furthermore, possible distinctions must be recognized between the effects on plant growth of indoleacetic acid and auxin.

VAN OVERBEEK (3), by *Avena* coleoptile studies, has found that to some extent indoleacetic acid is inactivated by light, but to a lesser extent than is the case with auxin *a*. This fact may serve to account for some of the differences in responses of cotton plants under high and low illumination.

The inquiries on the comparative reactions of cotton plants to indoleacetic acid and boron had the work mentioned in the introduction as its background. Inasmuch as the evidence indicates some replaceability of boron by acetic acid and the importance of light and possibly of temperature in the reactions, a possible advance has been made in an understanding of the role of boron. A clue is thus provided as to why like amounts of available boron, both in culture solutions and it seems also in the field, may be effective to different degrees in different seasons with varied climatic conditions.

Summary

Experiments with young cotton plants show that indoleacetic acid will to some extent replace boron as an element essential to the growth of root, stem, leaf vein, and other leaf blade tissues. The results suggest that boron is essential to the formation of auxin in plants.

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LIGHT STABILITY OF AUXIN IN AVENA COLEOPTILES

W. S. STEWART[1] AND F. W. WENT

Introduction

Auxins *a* and *b* and their lactones, when extracted from plants, are not inactivated by light of wave lengths longer than 4000 A (**6, 16**). If a decreased growth of plants in light as compared with those in darkness is considered to result from inactivation of auxin (**2, 7, 10, 11, 12**), then wave lengths longer than 4000 A do inactivate auxin when it is inside the plant. VAN OVERBEEK (**9**) assumed that this growth decrease was due to decreased sensitivity of the growing cells to auxin, since in transport experiments destruction of auxin was not found. WENT and THIMANN (**17**) have suggested that light may inactivate only the bound and not the transportable auxin.

If this latter suggestion were true it would give a valuable argument in support of the hypothesis that auxin exists in different states inside the plant, namely, as a free-moving or transportable auxin, and as bound auxin (**17**). Free-moving auxin can be obtained from plants by placing the parts to be investigated with their basal cut surfaces on agar, when such free-moving auxin will diffuse into the agar and can then be quantitatively determined by the *Avena* test. The bound auxin comes out of the cells after killing them. It is obtainable by extracting them with organic solvents, for example, ether. This extraction presumably removes both free and bound auxin. To test this suggestion by WENT and THIMANN of differential inactivation by light of free and bound auxin, bound auxin was extracted from plant material grown in complete darkness and from comparable material after exposure to light. With the same kind of material, "transport" experiments with free-moving auxin were likewise carried out in light and in darkness. Precautions were taken to exclude all light, even the weakest red light, from the plants grown

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in darkness. This precaution was necessary as experiments (**13**) have shown that the orange darkroom light is sufficient to decrease auxin production 33 per cent in *Avena* coleoptile tips.

Material

It was first thought that *Raphanus* would be a good experimental plant to investigate the destruction of auxin inside the plant by light, but a growth inhibitor completely masked the auxin present (**8**), so that another experimental plant had to be chosen. Seedlings of Victory oats from Svalöf, Sweden, were therefore grown in the standard manner (**17**), with the exception that immediately after planting they were placed in complete darkness. Later, extracts with ether according to VAN OVERBEEK (**14**) were made. The test plants for the auxin determinations were grown with occasional orange light, as is usual for the *Avena* test.

Results

EFFECT OF LIGHT ON AUXIN DURING ETHER EXTRACTION

Results of preliminary experiments showed that during the course of an ether extraction the extracted auxin was not inactivated by light. In these experiments *Avena* plants were grown for four days in total darkness, at 85 per cent relative humidity and 25° C., in a mixture half sand and half peat moss. The mesocotyl, coleoptiles, and primary leaves were then extracted as four separate lots with peroxide-free ether while still in total darkness. Twenty-four hours later the ether from two of the lots was evaporated off in darkness while with the other two it was evaporated off in daylight. The auxin was taken up from the ether extract by evaporating the last 1–2 cc. of ether to dryness on hot 1.5 per cent agar. The agar was then tested for auxin by the *Avena* test.

To facilitate the ether extraction in the dark, a few modifications of the method of VAN OVERBEEK (**14**) were made. A well was made in the center of the bottom of a 125-cc. Erlenmeyer flask by blowing an opening in the bottom and fusing a shell vial (1.2×3 cm.) over it on the outside. When used in extractions, the desired volume of agar was placed in the well and the ether to be evaporated added. As the ether evaporated the extracted auxin was taken up

by the agar. It was found essential to wash down the walls of the flask several times with purified ether to prevent loss of auxin on the walls. After evaporation, the agar was removed from the well, solidified, and cut into small blocks for application to the *Avena* test plants. With these modifications it was necessary only to use momentarily a small point of dim red light when separating the ether from the water layer of the extracted material. The data from four experiments are given in table 1. No difference in the amount of

TABLE 1

EFFECT OF LIGHT DURING ETHER EXTRACTION OF AUXIN FROM AVENA COLEOPTILES; EXTRACT TAKEN UP IN 0.283 CC. 1.5 PER CENT AGAR IN ALL INSTANCES

Experiment no.	No. test plants per determination	Auxin extracted						Auxin in exposed plants as percentage in unexposed
		Unexposed			Exposed			
		Actual negative curvature (degrees)	Amount extracted (gm.)	Curvature per gm. (degrees)	Actual negative curvature (degrees)	Amount extracted (gm.)	Curvature per gm. (degrees)	
161	12	18.3	1.355	13.6	13.0	0.833	15.6	115
162a	12	16.0	0.843	18.9	18.7	1.054	17.7	93
162b	12	16.0	0.978	16.4	17.2	1.077	16.0	97
164	18	15.3	0.909	16.9	14.0	0.819	17.1	101
Average	.....	.....	.....	16.5	.....	.....	16.6	101

extracted auxin was found whether the ether evaporation was carried out in darkness (except for momentary use of the red light) or in diffuse white light. It was assumed therefore that there was no destruction of auxin when the ether extract containing auxin was handled in a laboratory receiving only indirect daylight.

EFFECT OF LIGHT ON ETHER-EXTRACTABLE AUXIN IN PLANT

The procedure of the experiments on the effect of light on extractable auxin was to make ether extractions of auxin from *Avena* coleoptiles exposed to light and from unexposed coleoptiles (con-

trols). The auxin thus extracted, containing both free and bound auxin, was measured by the standard *Avena* test.

In some experiments only the coleoptile was extracted while in others the coleoptile and the primary leaf were used. In some in-

TABLE 2

EFFECT OF LIGHT ON ETHER-EXTRACTABLE AUXIN

Experiment no.	No. test plants per determination	Exposure to light (seconds)	Volume agar for each extract (cc.)	Auxin extracted				Auxin in exposed plants as percentage in unexposed	Notes
				Unexposed		Exposed			
				Curvature (degrees)	Weight (gm.)	Curvature (degrees)	Weight (gm.)		
13a	12	1	0.3	5.9	1.089	5.2	1.170	82.2	Tip 1.5 cm. of coleoptile extracted; exposed to sunlight after removal from plant
19	24	1	0.5	10.8	1.202	12.7	1.715	81.0	Same as 13a
25	24	5	0.4	13.0	1.183	14.3	1.456	89.2	Same as 13a
8	12	5	0.3	6.5	0.782	4.9	0.841	69.8	Coleoptile and leaf extracted; tip cm. of plant extracted; exposed while on plant
14	36	10	0.4	9.5	1.211	9.1	1.705	67.7	Same as 13a
45	12	10	0.3	12.3	1.047	13.9	1.309	89.6	Same as 13a
36	20	10	0.5	5.5	1.496	11.1	2.727	109.0	Same as 13a
167	20	30	0.57	11.0	2.654	8.1	2.632	83.0	Same as 13a
8	12	30	0.3	6.5	0.782	5.1	0.865	70.8	Exposed while on plant
6	24	60	0.4	11.7	0.960	13.2	1.380	78.8	Tip 2 mm. of coleoptile removed; only coleoptile extracted; exposed while on plant
5	12	60	0.3	22.1	1.002	20.8	1.320	72.6	Tip cm. of coleoptile extracted; exposed while on plant
47	24	100	0.4	14.0	1.623	17.1	1.648	120.0	Same as 13a
8	12	600	0.3	6.5	0.782	5.3	0.839	75.1	Exposed while on plant
1	12	45 (min.)	0.4	8.3	0.720	8.8	1.430	53.0	Only coleoptile exposed to sunlight while on plant
15	24	10	0.5	10.5	2.348	9.7	3.300	64.5	Same as 13a, but exposed at 6000 ft. elevation
159	12	60	0.28	12.3	1.447	8.4	1.045	94.1	Mesocotyl and coleoptile extracted; exposed 1½ ft. from 60 watt Mazda bulb on 110 v. AC
160	22	60	0.57	10.9	4.216	7.9	4.100	76.0	Same as 159
Average								81.0±3.3	

stances the tip millimeter of the coleoptile was removed. Most of the plants extracted were grown as for the regular *Avena* test, illuminated during the first 36 hours of germination by a red light which inhibits growth of the mesocotyl. In experiments 159, 161, and 169 the plants were grown in complete darkness. In the first

experiments the coleoptiles were exposed to direct sunlight while they were still attached to the plant; in later experiments the coleoptiles were removed, placed on moist filter paper in a shallow glass dish, and then exposed to light. The material was weighed and immediately extracted. The controls in the dark in all experiments were always kept in a light-tight box directly beneath the plants being exposed to light, so that temperature conditions were nearly the same for both sets of plants. Data of fifteen experiments, summarized in table 2, show that light exposures as short as one second decreased the amount of ether-extractable auxin. The data are too variable to draw any further conclusions regarding the effects of various types of illumination (sunlight, electric incandescent light, etc.), but the fact that less auxin can be extracted from plants which have been illuminated with white light cannot be doubted, even though in two out of seventeen cases an increase was obtained. Statistically the decrease of extractable auxin is significant.

Effect of light on free-moving auxin

The auxin which diffuses from living plant tissues is presumably being transported through the plant and is spoken of as free-moving auxin. Light apparently does not decrease the amount of free-moving auxin in *Avena* coleoptiles, in contrast to its effect on bound auxin. Free-moving auxin is transported through *Avena* coleoptiles at a rate of 10–12 mm. per hour. Accordingly, with sections of *Avena* coleoptiles 5.25 mm. long, auxin applied at the top should move through them in approximately 30 minutes. Sections this length were used in the following experiments. These sections were allowed to stand with their basal ends on wet filter paper for at least 30 minutes and then were placed upright on 1.5 per cent agar blocks. On the tops of the sections agar blocks were placed. All the top agar blocks contained the same concentration of auxin *a* or *b*, which had been extracted from corn meal (**12**). (Auxin *a* or *b* naturally occurs (**4**) in *Avena* coleoptile tips.) The sections were kept in the dark, in petri dishes lined with moist filter paper. After 30 minutes they were exposed to sunlight for one minute and then allowed to remain in the dark for an additional 29 minutes. At the end of this time the amount of auxin in the lower agar block was determined in both the exposed plants and the non-exposed controls. Using this technique,

it was presumed that the auxin had just begun to diffuse out of the lower end of the section and was present throughout its entire length at the time of its exposure to light. Twenty-nine minutes later the auxin that was at the top of the section of the coleoptile at the time of its exposure to light would be moving out of the section into the lower agar block. Thus if there had been any decrease in the amount of the transported auxin, a comparison of this experiment with an unexposed, control experiment would be expected to

TABLE 3

EFFECT OF LIGHT ON FREE-MOVING AUXIN GIVEN AS DEGREES NEGATIVE AVENA COLEOPTILE CURVATURE

Experiment no.	No. test plants per determination	Curvature (degrees)	
		Unexposed	Exposed
171	11	4.5	5.2
	9	4.5	4.2
	10	4.8	4.3
	12	5.5	4.3
172	9	6.4	4.9
	9	3.6	4.0
	7	3.4	4.2
173	12	5.0	6.1
	12	6.8	6.9
	12	5.3	6.2
Average	.....	5.0	5.0

show less auxin passing through the section. This was not found to be the case (table 3). The same amount of auxin was present in the lower agar block in both the exposed and the unexposed sections. One experiment of this type was continued over 2 hours by exposing the same sections to light for one minute after $\frac{1}{2}$, 1, and $1\frac{1}{2}$ hours in the dark (experiment 173; table 3).

Discussion

These experiments show that in the *Avena* coleoptile the distinction between free-moving or diffusible auxin and the auxin inside cells, or bound auxin (**17**), is based on an actual difference in the condition of the auxin. The amount of auxin moving through an

Avena coleoptile section is not affected by light, whereas the ether-extractable auxin is partially inactivated even by short exposure to white light.

Since the ether-extracted auxin is probably a combination of free-moving and bound auxin, and free-moving auxin is light-stable, it follows that it is the bound auxin that is inactivated by light. This conclusion had previously been drawn from indirect experimental evidence. The experiments reported here give direct evidence, however, and as the determinations were all made on the same material, *Avena* coleoptiles, the reaction of the bound and free-moving auxin to white light could be compared. Such had not previously been the case.

KÖGL, KONINGSBERGER, and ERXLEBEN (**5**) and C. KONINGSBERGER (**6**) have shown that radiation below 4000 A wave length inactivates only the lactone form of the crystalline auxin *a* or *b*. The free acid and the lactone form of auxin *a* or *b* are stable in visible light. This lactone in concentrated alcoholic solution is in equilibrium with the free acid form of auxin *a* at a ratio of about 50 per cent lactone and 50 per cent free acid (**6**). In the plant, however, visible radiation, which failed to affect crystalline auxin or its lactone, causes an inactivation of bound auxin. This may indicate that in the plant there is another substance present, an activator, which is required before visible radiation can inactivate the auxin. This activator possibly would be a carotenoid (**1, 7, 15**).

From the foregoing considerations it seems evident that while the auxin is being transported, this activator is unable to function. It also seems evident that inactivation does not occur through the lactone form, as suggested by KÖGL and KONINGSBERGER. In this regard also the mean percentage inactivation which could be expected would be 50 per cent if all the lactone were destroyed; furthermore, since auxin and lactone are in equilibrium in solution, one would expect a rapidly increasing percentage inactivation if the illumination continued for 45 minutes or longer. This was not found (table 2). The experiments do not support the hypothesis that the inactivation of auxin in plants by light is directly by way of lactone; they suggest rather that auxin is inactivated by light when it is in combination with some cell constituent.

While these experiments show possibly for the first time by direct determination that the bound auxin inside the plant is inactivated by light, others had already found that light does not decrease the amount of transported auxin. VAN OVERBEEK (9) has shown that in light 106.5 per cent auxin is transported through sections of *Raphanus* hypocotyl sections, if transport in the dark is considered as 100 per cent. The data from tables 9 and 10 of DU BUY (3) give 95.5 and 97 per cent transport of auxin in the illuminated sections when compared with the dark sections. These figures, as our own, are so close to the values for sections in the dark (100 per cent) that the conclusion seems justifiable that light does not decrease the amount of free-moving auxin.

Summary

Auxin, once it has been extracted from plant tissues, is stable in light. As shown by ether extractions, 19 per cent of the auxin inside the plant is inactivated by sunlight or incandescent electric light (23.5 per cent inactivated, omitting negative cases). There is no corresponding decrease in the case of free-moving auxin.

This work was done at the California Institute of Technology, Pasadena, California.

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FLUORESCENT LAMPS AS A SOURCE OF LIGHT FOR GROWING PLANTS

(WITH ONE FIGURE)

A number of sources of artificial light have been employed in growing plants under controlled conditions. For various reasons, most of these have proved unsatisfactory. Carbon arc lamps give light of high intensity and good quality, but are costly to install and maintain. Other sources of light are poorly balanced spectrally for best growth, give off large quantities of heat, and usually necessitate the use of cooling systems when utilized in closed chambers.

We have known for some time that Dr. D. R. HOAGLAND (University of California) and Dr. F. W. WENT (California Institute of Technology) have been successfully using lamps of the fluorescent type in some of their experiments. Recently we have grown plants using 30-watt fluorescent Mazda lamps of both white and daylight types but of no other colors. These lamps were arranged so as to give an intensity of approximately 600 foot-candles at the leaf surface and were the sole source of light. They were operated 16 hours each day. For purposes of comparison, some plants were grown with ordinary winter daylight and others in winter daylight supplemented (beginning at 4:30 P.M.) by $9\frac{1}{2}$ hours of approximately 60 foot-candles of light from an incandescent filament lamp. Cabbage, cocklebur, corn, kidney bean, Biloxi soybean, tobacco, and tomato were used. Plants under the fluorescent light grew most rapidly, had sturdiest stems, shortest internodes, largest and most numerous leaves of the deepest green color, and greatest fresh and dry weights. In general they appeared more like plants grown in summer sunlight than did those plants grown under either of the other two conditions of illumination (fig. 1; see p. 716).

Since these lamps give off a negligible amount of heat, they can be placed very close to the experimental material. They are much more economical and efficient in operation than incandescent filament lamps, and can be variously arranged in kind, number, and position so as to give different qualities and intensities of light. All points considered, these lamps have been found the best source of artificial light for growth of plants when used as the only source of light or as a supplement in winter in regions where daylight is of inferior quality and low intensity.—AUBREY W. NAYLOR AND GLADYS GERNER, *Department of Botany, University of Chicago.*

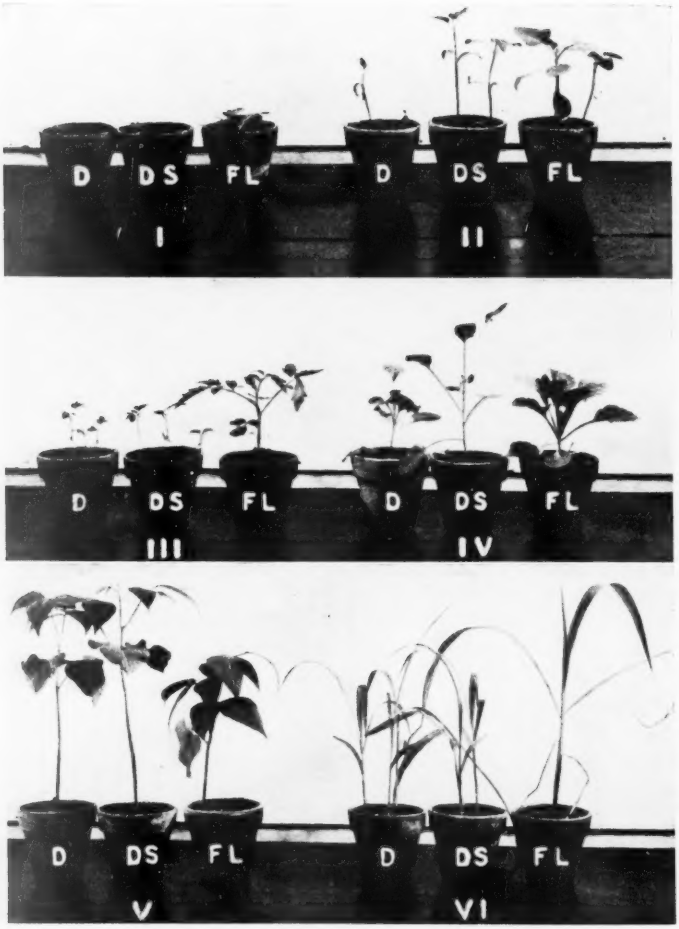

FIG. 1.—Response of plants to different types of illumination. *D:* ordinary winter daylight; *DS:* ordinary winter daylight supplemented by 0½ hours of incandescent filament light of 60 foot-candles intensity; *FL:* fluorescent light of approximately 600 foot-candles intensity for 16 hours per day as sole source of illumination. I, tobacco; II, cocklebur; III, tomato; IV, cabbage; V, kidney bean; VI, corn.

CURRENT LITERATURE

Les Fusarium et cylindrocarpon de l'Indochine. By FRANCIS BUGNICOURT. Encyclopédie mycologique XI. Paris: Paul Lechevalier, 1939. Pp. 206. Illustrated. 165 fr.

Fusarium and its fairly recent segregate *Cylindrocarpon* constitute a group of fungi of great importance, as well as one of great difficulty. The perfect stages of all species, so far as known, are members of the Hypocreales, but even if all were known, it would still be essential to consider the imperfect stages, the aspect under which these forms commonly present themselves in nature and in culture. The present volume gives detailed descriptions, cultural characteristics, and illustrations of some forty species and varieties of the two genera, isolated from various substrata in Indo-China over a period of 8 years, of which two varieties of *Fusarium* and nine species of *Cylindrocarpon* are described as new.

Every species and variety treated is illustrated by outline drawings of micro- and macroconidia and chlamydospores, if present; photomicrographs of most of them are reproduced on the six collotype plates and growth characters used in classification are fully shown on the four colored plates. The classification of *Fusarium* follows the well-known treatment of WOLLENWEBER and REINKING, the species included representing nine of the sixteen sections of those authors. It is unfortunate that the original numbering of the sections is not retained, while the lack of a complete index, including synonyms, is to be regretted. The present work is a useful supplement to the standard monograph mentioned, particularly as extending our knowledge of tropical pathogenic fungi and their host relationships.—G. W. MARTIN.

Growing Plants in Nutrient Solutions. By WAYNE I. TURNER and VICTOR M. HENRY. New York: John Wiley & Sons, 1939. Pp. xiv+154. Figs. 29. $3.00.

The popularity of solution culture gardening among amateurs has led to the publication of many books dealing with this pastime. As a commercial method of plant production, especially for greenhouses, solution culture offers a number of advantages which offset the original expense of installation of the necessary machinery. It seems probable, therefore, that commercial nutrient solution culture will gradually come into its own, while the popular fad is bound to decline.

For those who contemplate the conversion of greenhouses to the nutrient culture technique, this book offers much valuable information. The general

principles are discussed, the commercial advantages, and the construction changes required to convert a greenhouse from soil to the new method. Directions are given also for small-scale experimental units for those who may profit from preliminary investigations of their plant nutrition problems. Information required for the intelligent preparation of nutrient solutions, mathematical computations, sources of needed materials, and accepted formulae are presented simply and clearly. The book discusses the general physiological reasons for controlled culture methods; the problem of the essential elements and their functions in growth; methods of testing soils and foliage for mineral deficiencies; a key useful in diagnosis of deficiency symptoms; and the general cultural conditions to be met in successful commercial application of nutrient culture methods.

This work deserves to be classed among the better books dealing with solution culture methods. It is well written, fairly accurate, and does not misrepresent the subject nor attempt to exaggerate the importance of this new-old technique, which has been used by plant physiologists for nearly three-quarters of a century.—C. A. SHULL.

Submikroskopische Morphologie des Protoplasmas und seiner Derivate. By A. FREY-WYSSLING. Protplasma-Monographien, Band 15. Berlin: Gebrüder Borntraeger, 1938. Pp. x+317.

Applying morphological description to the molecular structure of protoplasm, FREY-WYSSLING has written an interesting and thought-provoking book. The domain of submicroscopic and molecular structure in biology is shown to fit between the cell theory and the atomic theory. It bridges the gap between the descriptive morphology of the cytologist and the atomic and molecular concept of the physicist.

As a foundation for the submicroscopic morphology of the protoplasm, the author presents concise discussions of: the phase theory and its relation to colloidal chemistry; the structural theory of atoms and molecules—their dimensions and spatial orientations as determined by X-ray diffraction measurements and their physical properties resulting from these characteristics; and the micellar theory. In the discussion of the micellar theory, gel structure is discussed at length, particularly as it is revealed by polarized light studies. Applying these theories to protoplasm, a description of the molecular morphology is conceived.

In the discussion of the cytoplasm, a "cohesion point" theory is suggested to build together the protein, lipoid, and phosphatide building units into a structural entity. The nucleus is considered from a similar viewpoint. Chloroplasts and their structural building units are discussed. Double refraction phenomena and microscopic structure are used to develop chloroplast structure from a molecular standpoint. The remainder of the book is concerned with the submicroscopic structure of protoplasmic derivatives. These are classified

as either structural substances (cellulose, chitin, keratin, etc.) or as reserve materials (starch, protein, crystals, etc.).

Throughout, the physiology discussed is mainly that of plants, although there are sections on the molecular structure of muscle during muscle contraction; on silk; on keratin; etc.

This book is essentially an application to protoplasm of the same concepts applied by the author to the cell wall in his previous work, *Die Stoffausscheidung der höhren Pflanzen*. It is well illustrated with diagrams, pictures, and tables. It contains a very complete bibliography of references not commonly readily available to biologists.—W. S. STEWART.

The Microscope. By R. M. ALLEN. New York: D. Van Nostrand Co., 1940. Pp. viii+286. Figs. 82, pls. 72. $3.00.

The work is a generalized treatise, which outlines the history of the microscope, its gradual improvement with time, descriptions of modern instruments and how to use them most effectively. A chapter on preparation of material for microscopical examination applies mainly to materials other than plant tissues. —E. J. KRAUS.

A Manual of the Liverworts of West Virginia. By NELLE AMMONS. Notre Dame, Ind.: University Press, 1940. Pp. 164. Illustrated. $1.75. (Amer. Midland Nat. 23: 3–164. 1940.)

Although this manual covers a limited and artificial geographic area, its use in bryological studies will undoubtedly extend to all states surrounding West Virginia, and many others. Keys, partial descriptions, comments, and illustrations for 111 species representing 56 genera constitute most of the book. The characteristics of liverworts and methods for their collection and preservation are presented lucidly.

It is to be regretted that *Plagiochila virginica* Evans is listed as not treated in this manual (Appendix I). The type of this plant, together with the rest of Dr. MILLSPAUGH'S collections, is in the Herbarium of Field Museum, Chicago, where perhaps it may have been unavailable for many years. Citation of collectors with dates of at least the rarer species would have enhanced the value of the book.

Since the arrangement of genera is in accordance with the latest interpretations of a phylogenetic sequence, this manual is a very useful compilation of hepatics.—P. D. VOTH.